N. Scott Lynn, Ph.D.
Chaberska 57, Praha 8 – Kobylisy 182 51
+420 777 304 491 • nslynn@gmail.com

Ph.D. Thesis Review Report

Thesis by: Ing. César Rodríguez Emmenegger
Study program: F-4, Biophysics, Chemical and Macromolecular Physics
Title: Sensitive Layers for Optical Biosensors and Protein Chips

This thesis represents a well-rounded study of the development of anti- and non-fouling surface treatment procedures for use in optical biosensing platforms. The experimental work in this thesis compares and contrasts the preparation and utilization of (*i*) self assembled monolayers (SAMs), (*ii*) end-grafted polymers, (*iii*) polymer brushes, (*iv*) and polymeric betaines on a variety of surfaces in order to prevent non-specific adsorption of biological molecules contained in a variety of physiological solutions, focusing primarily on blood plasma.

This thesis has been assembled as two sections, the Ph.D. thesis as well as a series of appendices. The thesis section is divided in to a 14-chapter summary of the experimental work and is relatively void of experimental details and protocols. The appendix section contains the peer-reviewed portion of this thesis, including 5 research reports, 2 communications, and a single manuscript in the final stages of submission/publication. Although seemingly rushed and a bit scattered at times, the 14 chapters of this thesis review the work in the appendices in a manner that is easy to follow, where each chapter seemingly follows the natural timeline regarding the progression of this work. Chapter 1 is a well-written introduction and serves as a very nice review on the problem of surface fouling regarding biologically relevant molecules via non-specific interactions. Following this, chapter 2 clearly lays out the goals of the thesis, and chapter 3 provides an introduction to the polymerization and characterization methods. In addition to providing results pertaining to the use of different surface coatings as potential anti- and non-fouling surfaces (and what components of a physiological solution are most problematic), chapters 4-10 present new challenges to the previously accepted ideas of what would constitute an ideal non-fouling surface. These chapters also present the use of novel, non-fouling surfaces that remain effective for a variety of physiological fluids, including 100% blood plasma. Chapters 11 and 12 extend the use of these surface coatings to non-gold surfaces and nanoparticles, respectively, and chapter 13 provides a nice summation of the use of these coatings regarding real applications, including the direct detection of *cronobacter* bacterium in milk samples as well as the direct detection of a variety of Epstein-Barr virus (EBV) reactive antibodies in diluted blood serum. There are a number of highlights associated with this work, many of which are of great value to the scientific community:

1. The results contained in this thesis have successfully challenged several aspects related to the design of an "ideal non-fouling coating." These challenges include: (A) the use of single protein solutions (fibrinogen, IgG, lysozyme, etc.) for the characterization of a surface as being anti- or non-fouling is not acceptable. This thesis clearly demonstrates that surfaces existing as anti-fouling for a multitude of single protein solutions *does not guarantee* that the same surfaces will remain anti-fouling for complex physiological solutions. (B) the previously accepted conditions for an anti-fouling surface (hydrophilic, electroneutral, a surplus of hydrogen bond acceptors, minimization of hydrogen bond donors) are not an acceptable as a "stand alone" list, as the design criteria for non-fouling surfaces is much more complicated.
2. The work in this thesis also presents several coatings that remain non-fouling for an extremely wide variety of complex physiological solutions. These coatings, poly(CBAA) and poly (HPMA), provide full resistance (below detectable limits) to the non-specific adsorption of the components related to 100% blood plasma and serum, cerebrospinal fluid, saliva, urine, fetal bovine and calf serum, chicken egg, and whole cow milk. These results are very impressive, and represent the state of the art of non-fouling surfaces. In addition, these coatings were activated with several biocomponents on an optical sensing platform for use in a variety of biosensing detection experiments (Ch. 13).
3. The anti- and non-fouling coatings detailed in this thesis were primarily based on gold surfaces utilizing the formation of SAMs via the gold-thiol interaction. This thesis also presents the use of these coatings on a variety of surfaces using plasma sputtered nylon (PSN). This technique allows for the use of non-fouling coatings in a variety of relevant applications, including (non-plasmon based) biosensing applications, drug delivery processes, and biomechanical implants.

This thesis has only a small amount of drawbacks, which are detailed below. It should be noted that these drawbacks are relevant to the text in the thesis, and do not take away from the experimental work performed therein.

1. There are consistent citations of references that are not appropriate for the respective text. A single example of this can be found in reference [11] in chapter 3, where there is seemingly no background information pertaining to pseudo-living polymerization techniques.
2. There is a large amount of material in this thesis dedicated to the use of pseudo-living ATRP techniques, including the bulk of chapters 6 and 8. Despite the prevalence of surfaces prepared by this technique, this thesis contains only a relatively small background on the subject (Ch. 3). Compounding this lack of introduction, the material in chapter 3 is somewhat scattered and difficult to read, and the reader is forced to resort to the appendices.
3. There seems to be a multitude of problems associated with the figures and tables in this thesis. These problems include: (*a*) figures not cited in the text at all (C5-2, C9-3, C11-7), (*b*) the reader is provided very little information concerning the data in the figures (Figures C4-4, C6-4, C8-3; Tables C7-1), or (*c*) the figures are labeled in such a manner that makes them somewhat difficult to decipher (Fig. C4-5).
4. There is a persistent use of ambiguous and/or passive phrases within the thesis. Examples are (*i*) Page 6-5: "An almost linear increase was observed…" (*ii*) Page 8-5: "…and probably led to disproportionation of Cu(I) generating some Cu(0)…" (*iii*) Page 9-4: "The detection of analytes by optical biosensors is in general fast…" (*iv*) Page 11-4: "…within the standard deviation of the method…"

In addition, for discussion purposes, I suggest one or more of the following issues to be discussed by Ing. Rodríguez Emmenegger at his defense:

1. The bulk of this thesis involves the synthesis of non-fouling coatings for use in biosensing applications. In these applications, one of the most important parameters involves the available active surface binding site density C_B, where at each active site an analyte present in solution has the potential to be captured via an affinity interaction. Despite its importance, (outside the detection of DNA/RNA oligiomers) experimental measurements of this parameter remain, at best, very

difficult to measure. Using the novel surfaces discussed in this thesis, is there a convenient way to both measure and/or control the density of active sites?

2. The coatings poly(CBAA) and poly(HPMA) were shown to be completely resistant to fouling from a wide variety of physiological solutions. Of course, on their own these coatings will not function as a biocomponent for biosensing applications. Are these coatings expected to remain non-fouling after these coatings have been further activated with biocomponents such as IgG, DNA/RNA, aptamers, etc.?
3. The surface independent approaches (Ch. 11) to obtain non-fouling surfaces are very important for a wide variety of applications. For biosensing applications, there are many components of the sensing device outside of the transduction/sensing area that also require a non-fouling surface. Despite their importance, these components (tubing, inlet ports, storage vials) are rarely discussed and are for the most part ignored as a sink for non-specific adsorption of relevant analyte. Using the results of this thesis, are there approaches where these coatings can be applied to the inner surface of a generic tube (PEEK, polypropylene, Silicone…) possessing a very large length/radius aspect ratio?

To conclude, it is my opinion that the results in this thesis are above and beyond the requirements for a doctoral degree. I hereby recommend that the dissertation prepared by Ing. Césare Rodríguez Emmenegger, entitled "Sensitive Layers for Optical Biosensors and Protein Chips," be accepted as fulfilling in part requirements for the degree of doctor of philosophy.

Nicholas Scott Lynn Jr., Ph.D.

Postdoctoral Fellow
Institute of Photonics and Electronics
Academy of Sciences of the Czech Republic
Chaberska 57, Prague 8 182 51
Czech Republic