Dear Dissertation Committee,

I am writing this letter to provide feedback on Ivana Visova's dissertation, titled "The Study on Interactions of Functional Surfaces with Biological Systems." In her thesis, Ivana discusses a range of studies on novel anti-fouling coatings for preventing nonspecific adsorption of molecules (especially of a biological origin) on surfaces. Ivana covers both the theoretical background of surface adsorption, as well as the experimental techniques employed, before discussing her original work across several related but conceptually distinct projects. In short, this is an excellent thesis, and there is no question that Ivana has carried out original, interesting, and highly impactful work in antifouling coatings and their potential application to real-world problems. Her dissertation is well-written, interesting, clear, and the progress from basic scientific questions to specific applications demonstrates an impressive breadth of scientific training and facility with multiple complementary experimental methods. Below, I will outline specific comments on each of the sections, and pose some questions for her dissertation defense. However, overall Ivana's work is impressive, and she undoubtedly deserves to pass her defense.

Introduction, Part 1, and Part 2. Ivana's introduction is very good overall, and she motivates the need for antifouling coatings well. The writing is professional and clear, and she outlines the scope of the dissertation nicely. It should also be noted that she has clearly been highly productive during her PhD studies, with eight publications, and several more projects in the pipeline. The theoretical description of surface fouling and how to design non-/anti-fouling coatings is thorough and meticulous, and Ivana covers a great breadth of topics. Impressively, the introduction is easy for a non-expert in the field (like me) to follow, and she distills a number of complex topics into an understandable narrative that cover key points without getting unnecessarily bogged down in too many details. My own expertise is in bioconjugation chemistry and mechanisms for modifying proteins and peptides, so I can particularly comment on this section of Ivana's chapter. She covered an impressive range of techniques, with a critical analysis thereof, and I very much enjoyed reading about the challenges and opportunities for these methods in surface coatings. Ivana and Hana should consider publishing this section as a critical review of the field, if they are not planning this already. In terms of experimental methods, Ivana's description of SPR was nicely described, and once again I found myself learning a number of new aspects about this method. It was also nice to see a description of fundamental mathematical principles behind the processes.

Part 3: Results. This section of Ivana's thesis is of course the most important, and she nicely showcased the wide range of studies that she carried out as part of her PhD work. Below I will outline a few key points, both to highlight interesting portions of her work as well as to raise a few questions for future discussion:

- The description of the polymerization process and the monomers used is very clear, and the new experimental setup devised for SI-ATRP is an interesting and impactful contribution to the field.
- The lack of NHS ester hydrolysis on the surface was surprising, given that these molecules are (as Ivana pointed out) usually unstable in aqueous solution beyond a few hours. Is it known why they seem to be so recalcitrant to hydrolysis? Does the surface play a role in structuring the water and/or preventing its access? What else is known about this process?
- The systematic study of molecules to promote NHS ester hydrolysis was quite interesting; did the conclusion of which molecules worked best help provide insights into the degradation resistance (as mentioned above)?
- Overall, Ivana used a range of characterization methods to probe the surface properties from many different perspectives. This use of complementary but mechanistically distinct methods strengthens the conclusions significantly.

- The multi-step SPR experiment—to probe the adsorption and washing, over several steps—is a nice way to probe various processes in surface adsorption that many experimentalists (including myself!) take for granted.
- There is an error in Figure 31: the product of the reaction with the NHS ester should be an amide, but the chemical structure shown is a ketone, with a primary amine dangling off the alpha carbon, instead of being attached directly to the carbonyl carbon.
- Another unexpected result is that the permanently charged (sulfonated) deactivating molecules work best at an intermediate ratio, and then the antifouling behavior decreases as the fraction increases past this. What are some plausible explanations for this switch in behavior?
- The linear tunability of the antifouling behavior with brush composition is very elegant, and makes for a very modular and programmable system.
- The progression from fundamental studies on swelling and deactivation, all the way to applications with real biological systems (and real-world problems) makes for a nice evolution of the thesis overall.
- It is interesting that the antifouling surfaces do not completely prevent fouling when exposed to cells; is there a hypothesis for why this occurs?
- Why does the OEG SAM not show a concentration-dependent RGD effect? Is it simply because the fouling of the surface enables cell adhesion regardless of the peptide?
- The results from the various “real-world” applications (bacteria-phage interactions, bacterial detection, miRNA detection, and SARS-CoV-2 targeting), and the breadth of systems that this works for, are quite impressive. However, why were these results not presented in the thesis more at length? I would have liked to see the primary data and a bit more discussion of the future potential/outlook for these coatings, as opposed to mentioning the results and pointing the reader to a publication for the full story.

Overall, my impressions of Ivana’s dissertation are highly positive. Her work is interesting, innovative, well-motivated, and clearly-presented. Ivana has done a significant amount of original work, from fundamental physics investigations to novel chemical system and practical applications, and her research will open the door to a range of future investigations on this topic. I myself learned a great deal from reading the dissertation, and am excited to see what her future research brings, and what new innovations these materials spur in the field.

Sincerely,

Nicholas Stephanopoulos, PhD
Assistant Professor, School of Molecular Sciences
Biodesign Center for Molecular Design and Biomimetics
Arizona State University