MICRO- AND NANOTECHNOLOGY-BASED PLATFORMS TO STUDY BIOLOGY AT SMALL SCALE: FROM DNAS TO SINGLE CELLS

Thesis by

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In Partial Fulfillment of the Requirements for the

degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2011

(Defended March 11, 2011)

To my family,

Jinha and Seung-Hyuck

Acknowledgements

I have been so fortunate to interact with talented and intelligent people during my stay at Caltech. First of all, I would like to thank my advisor Professor Jim Heath. His passion for science always motivates me to think deeply, and creatively, and to move forward. The academic freedom he gives and the resources he makes available to the group have extended my scientific/engineering experience and skill sets to the scope that I cannot imagine reaching in another place. I was lucky to work with great collaborators through Jim's organization of the Nanosystems Biology Cancer Center (NSBCC) between Caltech, UCLA, and the Institute for Systems Biology (ISB). I would like to thank many senior collaborators within the NSBCC, including Professor Raphael D. Levine, Professor Françoise Remacle, and Professor Paul Mischel. Discussion with them has always been fruitful and intellectually pleasing. I also thank the members of my thesis committee, Professor David Tirrell, Professor Morteza Gharib, and Professor Jack Beauchamp.

I have had the pleasure of working with many talented co-workers. Specifically, I have worked closely with Yuri Bunimovich, Michael Amori, and Heather Agnew on silicon nanowire biosensors, and Kiwook Hwang, Habib Ahmad, Qihui Shi, and Rong Fan on single-cell barcode chips. I have also worked with Hugh Kim in Jack Beauchamp's group and Hyungjun Kim in Bill Goddard's group on the lung surfactant project. Working with them has been a great rewarding and pleasure. I am very grateful to the current and past members of the Heath group. Especially I had wonderful time with Woon-Seok Yeo and Jang Wook Choi (and Tae Hyeon Yoo in Tirrell's lab) by sharing personal lives as well as

scientific ideas. I thank Kevin Kan for his effort to manage our lab facilities. I also must thank many Korean friends at Caltech with whom I could share and enrich my personal life with joy and happiness.

During the past six-and-a-half years at Caltech, I had the most dramatic change in my life. I got married to my beautiful wife, Katherine (Jinha), and my adorable son, Christopher (Seung-Hyuck), joined our family. I owe much to them for their unlimited support with love, understanding, and motivation. I thank my family members on both sides, especially my parents and parents-in-law, for their encouragement and support. Last, but by no means least, I would like to thank God, who has been so sincere and responsive to me all the time.

Abstract

This thesis describes technology platforms for various biological applications at nano- and microscale. The first platform is the silicon nanowire (SiNW) field-effect-transistor (FET)-based biosensor. SiNW FETs have unique features such as label-free, real-time, and electrical measurement, which will be demonstrated with DNA and protein sensing. We further demonstrate that using different surface chemistry can modulate the sensitivity and dynamic range of the sensor. Debye screening, one of the major bottlenecks of the technology, is shown to be circumvented by using electrostatically immobilized capture DNA for DNA sensing and a small synthetic capture agent, peptide, for protein sensing. A model for the detection of analyte by SiNW sensors is also developed and utilized to extract DNA binding kinetic parameters, which shows the potential of the platform as a more sensitive version of surface plasmon resonance (SPR).

The second part of this thesis focuses on a more practical and easily expandable technology, the microfluidics-based platform, to perform a single-cell-based protein analysis. We develop a flow patterning technology to generate highly parallel DNA barcodes that can be further utilized as a handle to immobilize protein capture agents, such as antibodies. As a first step, a protocol to make high-quality DNA micro-barcodes with an excellent uniformity is introduced. The uniform DNA barcode patterns enable us to perform protein detection from single cells in a microfluidic device that spans the whole glass microscope slide. A data set from about thousand experiments can be collected from a single test with the developed microfluidic device, owing to the good quality of DNA

barcodes and DNA Encoded Antibody Libraries (DEAL) technology. This platform further demonstrates that multi-parameter protein detection at the single-cell level presents cellular heterogeneity which leads to new findings in biology. A quantitative version of the Le Chatelier's principle, as derived using information theory, is applied to analyze a large amount of data from this platform. This principle provides a quantitative prediction of the role of perturbations and allows a characterization of a protein–protein interaction network.

Lastly, another application of microfluidics is demonstrated for studying interfacial chemistry on lung surfactant systems under oxidative stress, along with mass spectrometry (MS) and molecular dynamic (MD) simulation results. The findings from the MS and MD simulations provide mechanistic details for the reaction of ozone with unsaturated phospholipids, leading to possible damage of the pulmonary system by ROS or direct ozone exposure. These investigations focus on molecular transformations that occur as a result of oxidative stress. Such molecular transformations can have a strong influence on the physical properties of the pulmonary surfactant (PS) system (i.e., the surface tension and elasticity of the interface), and therefore understanding how chemical transformations influence such physical properties can provide key insights into how the PS system responds to environmental challenges. Thus, we also propose utilizing microbubbles as a model system for investigating the physical transformations of the PS system when exposed to environmental challenges. The chemical composition change, along with physical property change, is analyzed by altered bubble size and oscillatory behavior which can provide an improved understanding of the physics of a PS system when it is subjected to oxidative stress.

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