Chapter 5

Conclusion and Future Directions

This thesis analyzed the difference between traditional biochemical assays and real cellular environment, and identified three important differences: molecular crowding, low copy number, and spatial confinement. To reconcile these discrepancies, special treatment is needed in both theoretical and experimental works. On one hand, it is instructive to incorporate these features into the model of biochemical reaction networks to anticipate their influence. On the other hand, it is beneficial to be able to reconstruct the essential features of cellular systems in an in vitro study.

Chapter 2 discussed the implication of low copy number and spatial confinement using model systems. Both numerical and analytical approaches were employed. All the case studies in this chapter were motivated by realistic problems, and it would be interesting to directly test the predictions in experiments. Chapter 3 and 4 then described two microfluidic devices which may prove useful for this purpose. They enabled easy control of molecular crowding, copy number, and spatial confinement. As such, it is more biologically relevant to study the interaction among biomolecules using these devices. In addition, the devices reported here can be employed in the detection of biomolecules with less time consumption.

The experiments in chapter 4 also revealed the importance of shearing force in the redistribution of surfactant molecules and the resulting partial loss of interface protection. This in turn led to the inactivation of enzymes due to nonspecific adsorption to the interface. This influence was more significant when the droplet size was small because of higher shear stress applied as well as higher surface-to-volume ratio. Special attention is thus required to correctly interpret experimental observations in droplet-based microfluidic devices as their characteristic dimension is decreased. Future

work may involve direct recording of the transient process of surfactant convection and enzyme adsorption, and the quantitative analysis of the interaction among shear force, mass transport, and interfacial tension.