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Probing model systems by atomic force microscopy toward the comprehensive understanding of receptor-ligand interactions in biological environments

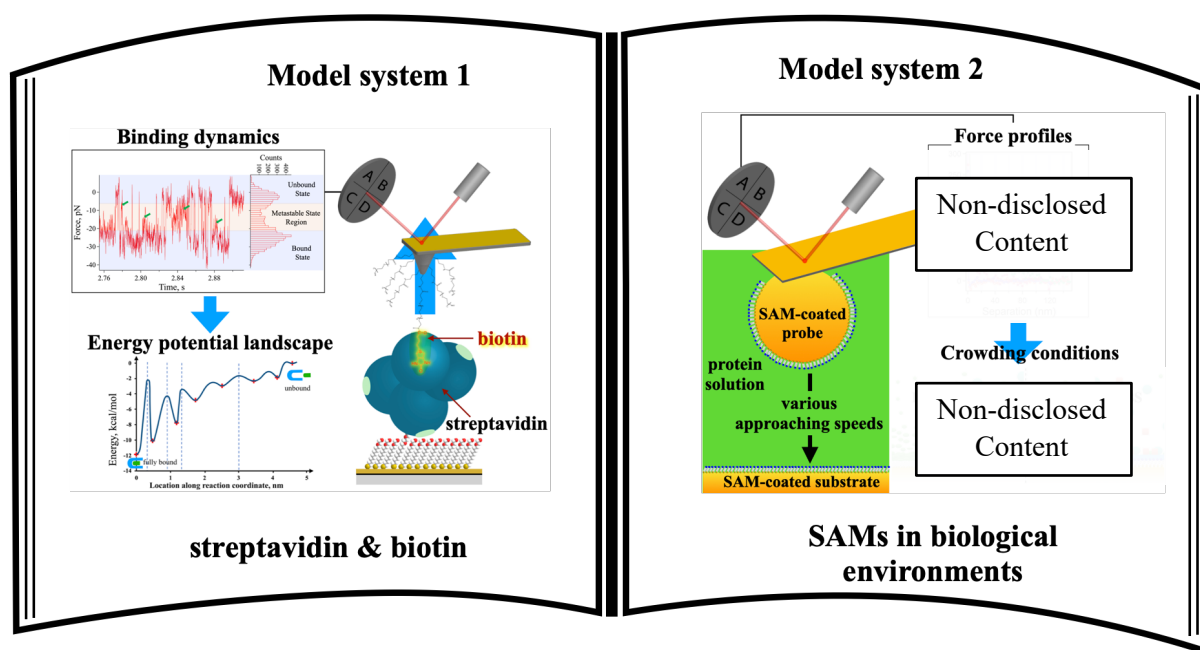
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Thesis Outline



Chapter I: Introduction

The molecular recognitions occurring between receptors and ligands are fundamental interactions in living bodies that determine cellular behavior and response. Due to this unique pairwise specific interaction, several receptor-ligand systems are highly explored into usage for biosensors and biomaterials operating in biological *in vivo* and *in vitro* conditions. However, the stochastic dynamics and the interplay of several interactions in biological systems have been evidently presenting a consequential amount of ambiguity. A biological environment is highly crowded with several species of biomolecules which affect the kinetics and stability of receptor-ligand interactions, as emphasized by numerous studies. However, in these studies, the quantification of the effects of crowding is carried out with the assumption of a homogenous crowding situation as that in a bulk medium. The crowding of molecules at the interfacial vicinity should be investigated since most of the active interactions in biosensors and biomaterials are situated at regions close to the solid support surface. As much as the underlying mechanism of receptor-ligand interactions is already interestingly complicated, the nature of biological environments adds up to the overall complexity.¹⁻⁴

Chapter II: Review of Related Literature

Atomic force microscopy (AFM) has shown superior capabilities in probing biological phenomena at the single-molecule and interfacial level to understand the mechanics and nature of interactions. AFM operates by monitoring the forces encountered by a cantilever as it approaches or retract from a sample. Through recent developments on probe functionalization and sample preparation through surface modification, detection of forces at pN-level and at more local regions than the bulk is rendered possible. These probed phenomena include the intermolecular dynamics of a specific bond and the macromolecular crowding effects at the interface.^{3,5-7}

Chapter III: Methodology

In this thesis study, a bottom-up approach was strategized through dissecting the overall complex process into fundamental units using model systems. By capitalizing on the capabilities of AFM, two measurement conditions were devised: 1) observation of the binding dynamics in high temporal resolution of a model receptor-ligand pair in an isolated buffer system to avoid the complexity of the multitude of interactions, and 2) investigation of the crowding scenario at the interfacial level as a consequence of the nature of biological environments using a model analytical platform by colloidal probe technique.

Chapter IV: Results and Discussion: Streptavidin-biotin system

For the model receptor-ligand pair, I used the streptavidin-biotin system as it is known to be the strongest noncovalent interaction occurring in nature, yet its binding mechanism is not fully understood. Through the investigation of this system's near-equilibrium dynamics by high temporal resolution atomic force microscopy under a buffer condition, I was able to generate a more detailed energy landscape of the system at the pulling reaction coordinate. In this energy landscape, four metastable states were revealed signifying the strong nature of binding, together with another two metastable states that are located beyond the binding pocket of streptavidin which may explain any nonspecific bindings that occurred in the measurements.

Chapter V: Results and Discussion: Crowded Biological Environment

On the clarification of nano-crowded environments at interfaces, two types of model surfaces were used based on self-assembled monolayers (SAMs): a protein-adsorbing from octanethiol (C8) and a protein-resistant from zwitterionic sulfobetaine thiol (SB), immersed in two types of biological media: bovine serum albumin (BSA) solution and fetal bovine serum (FBS). My key strategies were the employment of the colloidal probe technique to accurately quantify the detected forces, and the use of different approaching speeds to distinguish the mobile layer of biomolecules from the solid compact layer. Due to the hydrophobic nature of C8 SAM, a significantly more crowded condition than the bulk was produced in BSA that extended up to 130 nm from the SAM surface. The increase in crowding was presented by the increase in viscosity at the interphase region (5 times higher than the bulk) then to a more compact layer of denatured BSA molecules nearing the SAM surface. In FBS, the stability of large molecules that were deposited after attaining equilibrium prevented the formation of a viscous interphase layer. However, a thicker adsorbed elastic layer is present. For SB SAMs in both crowding media, there is no significant discrepancy in the crowding state between the bulk and interfacial regions based on the force profiles and rheological parameters. This is attributed to the presence of interfacial water at the zwitterionic terminal groups that prevented the aggregation of biomolecules.

Chapter VI: Conclusions and Overall Outlook

Although several implications can already be deduced on the effect of the different crowding scenarios on the kinetics and equilibria of the molecular recognition between streptavidin and biotin based on the known crowding effects (e.g. volume exclusion and hydrodynamic interactions), further experiments have to be performed to provide straightforward evidence on the possible profound effects of crowding especially regarding densely packed confined regions on fouling surfaces. Finally, I expect that the measurement and analytical techniques designed in this study, and the findings obtained using the model systems can be well extended and applied to a wide variety of receptor-ligand systems on diverse types of surfaces operating under biological environments. Our overall approach mediates top-down and bottom-up strategies on the research of macromolecular crowding, addressing several issues in the field of biomaterial and biosensor design.

Major References

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