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Hydration states of ionic liquids stabilizing protein structures and evaluation of proteins' activity on model organic surfaces

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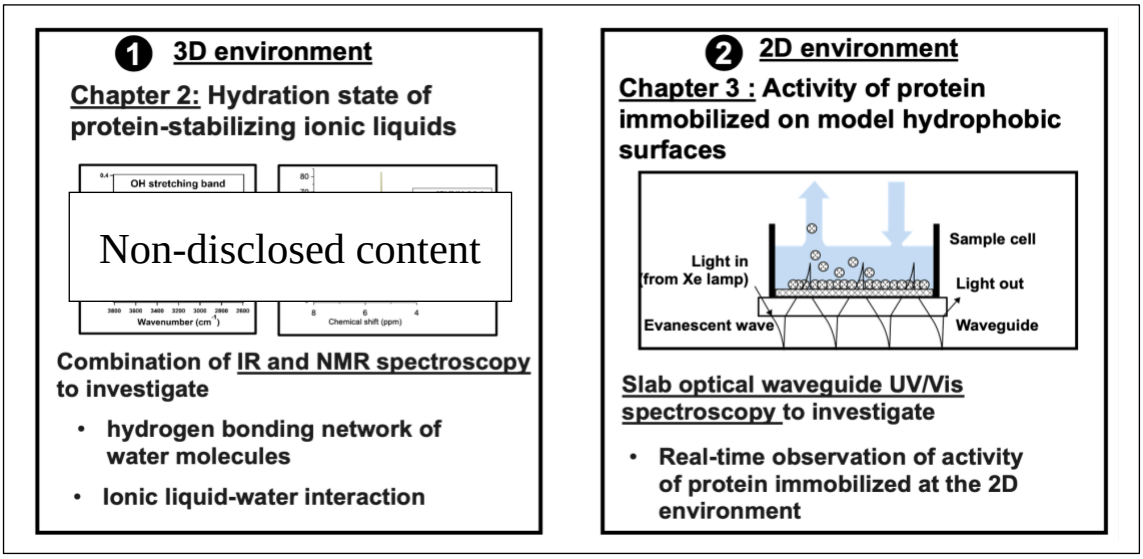
Thesis Outline



Chapter 1: Introduction

Protein-material interaction in the two-dimensional (2D) environment and three-dimensional (3D) environment is completely different. A 3D environment refers to the protein material interaction in a solution, and 2D environment refers to the protein material interaction at the interface between solid and liquid phase. In a 3D environment, protein molecules simultaneously interact with water molecules and excipient molecules, such as salt, sugar, and polymers in a highly competitive manner. However, in the case of a 2D environment, the protein-material interaction is limited to the boundary between the solid surface and the bulk liquid. Understanding the protein-material interaction in 2D and 3D environment is essential for the development of protein based biomedical applications, for instance, drug delivery systems biomedical implants, biosensors, etc.

This thesis discusses the strategies to investigate the protein material interaction in 2D and 3D environment using cytochrome c (cyt c) as a model protein. In chapter 2, I investigated the hydration state of protein-stabilizing ionic liquids (ILs) (3D environment) using an IR-NMR combinational approach. In

chapter 3, I investigated the activity of protein immobilized on model organic surfaces (2D environment) using a slab optical waveguide (SOWG) UV/Vis spectroscopy.

Chapter 2: Investigation of the protein stabilization capability of hydrated ionic liquids (Hy ILs) using attenuated total reflectance infrared (ATR-IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy

Certain hydrated (Hy) ILs have shown superior protein stabilization capability, for instance choline dihydrogen phosphate IL when hydrated with 20 wt.% water showed the capability to stabilize cyt c, lysozyme, and concanavalin A.[1–3] In this chapter, I investigated the molecular interaction in Hy ILs required to achieve the protein stabilization capability using attenuated total reflectance (ATR) IR spectroscopy and proton (^{1}H) NMR spectroscopy. I observed that the presence of less perturbed hydrogen bonding network of water molecules in the ion hydration shell and strong electric field effect of the ion pair is responsible for the protein stabilization capability of Hy ILs. Furthermore, I also demonstrated that the IR-NMR combinational approach is an excellent probe for investigating the electric field effect of Hy ILs.

Chapter 3: Real-time observation of protein activity on organic surfaces using slab optical waveguide ultraviolet-visible (SOWG-UV/Vis) spectroscopy

Investigation of the activity of proteins molecules immobilized at the solid-liquid interface is complicated due to the lack of surface sensitive and selective techniques.[4,5] Using SOWG UV/Vis spectroscopy can circumvent the complications in real-time observation of proteins immobilized at the solid-liquid interface. In this chapter, I investigated the activity of the cyt c protein molecules immobilized on hydrophobic self-assembled monolayers (SAMs) such as octadecyl silane (ODS) SAM. Trichloromethyl silane (TCMS) SAM and ODS-TCMS mixed SAM. Furthermore, the activity of cyt c was observed on hydrophilic bare glass as a control. I observed that on bare glass, the cyt c immobilized without any change in the activity. However, on hydrophobic SAMs, the immobilized cyt c underwent a change in the redox activity; the immobilized cyt c were oxidized at the solid-liquid interface. One possible explanation for this oxidation could be due to the oxygen molecules trapped in the nanobubbles formed on the surface of the hydrophobic SAMs, which when interacting with immobilized cyt c molecules results in oxidation.[6]

Chapter 4: Conclusion

This thesis answers the following questions: “Why certain Hy ILs stabilize protein structures?” (Chapter 2) and “what is the activity of cyt c protein molecules immobilized on hydrophilic and hydrophobic surfaces?” (Chapter 3). In a 3D environment, the pure water-like hydrogen bonding network of water molecules in the ion hydration sell is essential for mediating the interaction with the protein molecules. The hydration shell around the ions creates a microenvironment that protects the structure and the function of protein molecules. However, in 2D environment, the formation of nanobubbles on the hydrophobic surfaces could be a possible reason for achieving the change in the redox activity of the protein immobilized at the solid-liquid interface. In this thesis, the observations made from protein-material interaction in 2D and 3D environment can contribute to the development of IL-based drug delivery systems and hydrophobic coatings for biosensors and medical implants.

Major References

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