学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（理学）　氏名　Wen Xinxin

学 位 論 文 題 名

Study on Inter-Protein Electron Transfer as Rate-Limiting Steps in Microbial Electron Conduction
（バイオフィルムの電子伝導における律速段階としてのタンパク質間電子移動に関する研究）

Microbial biofilms, particularly those formed by electroactive bacteria, facilitate long-distance electron transfer (LDET) across spatially heterogeneous environments, enabling key applications in bioenergy, bioelectronics, and pathogenesis. Although substantial progress has been made in characterizing the structural components of these biofilms, the mechanistic bottlenecks that constrain their electron conduction remain poorly understood. This dissertation systematically investigates the molecular, biophysical, and thermodynamic principles governing two principal conduction pathways, inter-cytochrome electron transfer and vesicle-mediated electron transport, in both model electroactive and pathogenic biofilms. Special emphasis is placed on the role of extracellular redox-active flavins, which dynamically shuttle electrons as redox cofactors to regulate conduction efficiency and pathway selection in diverse biofilm systems.

Chapter 1 provides an overview of the research background.

Chapter 2 investigated the mechanism of electron conduction in *Shewanella oneidensis* MR-1 biofilms, a model electroactive bacterium, with particular emphasis on interprotein electron transfer. While previous studies proposed that electron hopping between hemes 5 and 10 in the outer membrane cytochrome MtrC is the rate-limiting step, the strong intramolecular coupling between hemes suggests that interprotein interactions may constitute the true bottleneck. To test this hypothesis, temperature-dependent conduction currents were measured using indium tin oxide (ITO) interdigitated electrodes in both wild-type and mutant strains. Deletion of periplasmic cytochromes had little effect on activation energy (E_a), whereas deletion of omcA or mtrC led to a threefold increase in E_a, indicating that electron transfer between OmcA and MtrC is critical for efficient conduction. Additionally, reduction of outer membrane fluidity significantly increased Ea, while inter-heme exciton coupling remained unchanged, highlighting the importance of protein diffusion. Notably, flavin binding suppressed heme-based conduction but enhanced flavin-mediated currents, likely by obstructing key interprotein transfer sites at hemes 2 and 7. These findings establish interprotein electron transfer and flavin modulation as key determinants of biofilm electric conduction, laying a foundation for engineering high-performance bioelectronic materials.

Chapter 3 explored the role of pathogen-derived outer membrane vesicles (OMVs) in mediating electron transfer within the oral biofilm of *Porphyromonas gingivalis* (PG), aiming to translate the principles established in **Chapter 2** into a pathogenic context by investigating the conductive properties of PG biofilms. While OMVs are known to support bacterial survival by promoting biofilm formation, densely packed

microbial communities often experience metabolic inhibition under reductive stress. Evidence showed that OMVs facilitate extracellular electron transfer, thereby sustaining metabolic activity in reductive biofilm environments. Using interdigitated electrodes with 10 μm spacing, a fivefold increase in conduction current (I_{cond}) was observed when biofilms were incubated with isolated OMVs. These vesicles were enriched in peptidylarginine deiminase (PPAD), an enzyme with citrullination activity. Genetic knockout or chemical inhibition of PPAD significantly reduced the I_{cond} enhancement, whereas supplementation with FMN, the cofactor of PPAD, further amplified the conductive response. Moreover, PPAD-containing OMVs markedly promoted biofilm formation on calcium phosphate discs, indicating that vesicle-mediated electric conduction is a key factor in pathogenic biofilm development. These findings reveal a novel electrochemical role for pathogenic OMVs and suggest new targets for anti-biofilm strategies.

Chapter 4 investigated the functional implications of redox-active biofilms in antibiotic resistance, building on the novel electrochemical role and mechanism of pathogenic OMVs identified in **Chapter 3** to explore new targets for anti-biofilm strategies focused on disrupting bioenergetic pathways. Redox-active OMVs were shown to contribute to antibiotic resistance in PG biofilms by maintaining redox homeostasis under drug-induced stress. While traditional antibiotics were less effective against OMV-rich biofilms, metabolic inhibitors such as nitazoxanide remained broadly effective, suggesting that targeting bioenergetic pathways can circumvent redox-based antibiotic tolerance.

Chapter 5 presents the overall conclusions and prospects based on this body of work.

Collectively, these findings establish flavins and redox proteins as key mediators of biofilm electron transfer, with broad implications for microbial respiration, pathogenicity, and therapeutic intervention.