Abstract

Thesis Title: Engineering of Synthetic Bioreceptors for Rapid Detection of *Staphylococcus aureus*

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Staphylococcus aureus is a significant public health concern, known for its wide range of virulence factors and increasing resistance to antibiotics, particularly within the ESKAPE pathogens group. Despite being a commensal microorganism, *S. aureus* can lead to severe infections, varying from soft tissue infections to life-threatening conditions like sepsis and endocarditis. The occurrence of methicillin-resistant *S. aureus* (MRSA) has further complicated treatment, necessitating the development of accurate, rapid, and cost-effective diagnostic tools. This thesis focuses on the design and engineering of novel bioreceptors, including molecularly imprinted polymers (MIPs) and DNA aptamers, for the detection of *S. aureus*, specifically targeting its Protein A and Clumping Factor A (ClfA). Through *in silico* molecular docking, MIPs were designed and optimized to ensure high affinity, specificity, and stability. Using *in silico* docking, monomers were rationally selected for MIP synthesis, optimizing their interactions with these target proteins (Protein A and ClfA). Multi-monomer MIPs was successfully synthesized, demonstrating high binding capacity (BC) and specificity for target proteins, with dissociation constant for Protein A-binding MIP and ClfA-binding MIP to be 0.22 and 0.51 μM, respectively. Alongside, DNA aptamers with high affinity and specificity for ClfA were selected using the Ni-NTA affinity SELEX process. These aptamers exhibited a dissociation constant of 0.27 μM, indicating a strong binding affinity. Comparisons made from the performance of MIPs and aptamers, exhibited distinct advantages for each approach. The ClfA-targeted MIPs, while demonstrating a slightly higher dissociation constant or a relatively lower binding affinity, are highly stable, making them particularly effective in scenarios where robustness and multi-point interactions are crucial. In contrast, the ClfA-targeted DNA aptamers, with their lower dissociation constant, offered superior specificity, which is advantageous in applications requiring precise molecular recognition and minimal cross-reactivity. Future directions for this research include leveraging advanced computational tools, such as machine learning and AI-driven modeling, to further enhance the rational design of MIPs. Additionally, the combination of MIPs and aptamers into hybrid platforms, along with the development of multiplexing capabilities, could significantly advance the field of bacterial diagnostics. These innovations aim to provide comprehensive, real-time detection of

multiple pathogens, thereby contributing to more effective management and treatment of bacterial infections.