Protein-based Approaches for Biosensor Development.

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Introduction: Biosensors are critical tools for detecting and processing biological signals, with applications in environmental monitoring, medical diagnostics, and bioengineering. The current study investigates two distinct approaches for biosensor module development: protein-based and cell-based systems. This research focuses on engineering bacterial two-component systems (TCS) and developing directed evolution platforms to enhance biosensor performance. The goal is to improve the specificity and efficiency of biosensors, particularly in detecting small molecules and facilitating rapid biological information processing.

Methods: We utilized thiosulfate-sensing (thsS/thsR) and tetrathionate-sensing (ttrS/ttrR) TCS from S. halifaxensis and S. baltica, respectively. These are being evolved to detect small molecules like perchlorate and aromatic compounds such as TNT through binding pocket analysis and rational design. We're using an error-prone PCR system and selective conditions (kanamycin for positive selection, sucrose for negative selection) to evolve TCS for improved selectivity towards new ligands. We focused on utilizing the non-conventional cofactor 3'-NADPH. Enzymes are being engineered to use 3'-NADPH through a cycle involving split AvrRxo1 generation and the introduction of mutations to enhance enzyme specificity. We plan on using PACE to continuously evolve biosensors, ensuring rapid adaptation to new environmental stimuli.

Results: We successfully evolved our TCS to detect perchlorate with enhanced specificity. Key mutations that improved ligand binding and signal relay pathways were identified. We optimized growth conditions and selective pressure, resulting in high sensitivity and specificity towards perchlorate strains. Analysis of selected colonies showed consistent mutations that enhanced detection capabilities. We developed a functional in vivo cycle for 3'-NADPH utilization. Key residues critical for enzyme specificity were identified and mutated, significantly improving the performance of the biosensor modules. We demonstrated the compatibility of TCS proteins with PACE cells, validating the continuous evolution approach for biosensor optimization.

Conclusions: The study presents significant advancements in biosensor module development using both protein-based and cell-based approaches. Engineered TCS and metabolic pathways exhibit improved specificity and faster response times, highlighting their potential for various applications. Future work will focus on further refining these systems and exploring their practical applications in real-world scenarios.

Acknowledgments: I'd like to thank Professor Scott Banta and the Banta Lab for hosting me this summer, and I express my specific appreciation to Samuel Lim, Ph.D., and Piyush Kumar for working with me.

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