REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Developing Yeast Whole-cell Biosensor For Potential Pathogen Detection

Tek Hyung Lee Lawrence Livermore National Laboratory **Feliza Bourguet** Lawrence Livermore National Laboratory **Salustra Urbin** Lawrence Livermore National Laboratory **Nichloas Be** Lawrence Livermore National Laboratory **Mimi Yung** Lawrence Livermore National Laboratory

Early and precise detection of pathogenic infection at the point-of-care (e.g., battlefields, rural communities) is critical for effective drug treatment and disease spread monitoring (1). While portable molecular-based biosensors (e.g., lateral flow assays) have been developed for sensitive pathogen detection, microbial whole cell-based biosensors have also drawn significant attention as another novel detection device. These biosensors are advantageous because they can detect target analytes with high sensitivity, but do not need expensive and time-consuming protein (antibody) purification steps required for molecular-based biosensors (2). In addition, the microbial biosensors can provide important information on sample cytotoxicity (3).

Among various microbe candidates for biosensor development, yeast is an ideal organism since it is safe and has well-developed genetic modification tools. Moreover, it can provide an inexpensive supply chain, and can be dried out for long term storage and rehydration (4). Previously developed yeast biosensors showed their sensitive detection capability, but the diversity of targets is highly limited (3, 4). A biosensor that can be readily adjusted to detect newly discovered disease biomarkers and emerged pathogenic antigens is in urgent need as an inexpensive point-of-care diagnostic device to prepare for future pandemics or potential biological warfare agents.

Toward developing a versatile yeast biosensor, we designed synthetic membrane receptors in yeast to sense a small molecule ligand. This synthetic receptor consists of i) a single-domain antibody, so called “nanobody,” as a signal sensor that tightly binds to the ligand (5), ii) a “splitubiquitin yeast two hybrid system” as a signal transducer that turns the nanobody-ligand interaction signal to a reporter (6), and iii) a reporter for signal readout. The nanobody in the receptor can be easily interchangeable with other nanobodies to interact with different ligands,
providing modularity to the yeast biosensor.

As a proof-of-concept study, we constructed a synthetic membrane receptor that detects a small molecule caffeine using previously established yeast transmembrane domain and caffeinetargeting
nanobody (7, 8). Our biosensor responded to a broad range of external caffeine concentrations (from nM to mM) with a highest signal output

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