ACS PRF NARRATIVE REPORT

1. **PRF** #57850-DNI4

2. **PROJECT TITLE** Expanding the scope of asymmetric transformations catalyzed by promiscuous biocatalysts in non-aqueous media

3. **PI**: Dr. Robert M. Hughes, Department of Chemistry, East Carolina University

4. No Co-PI

I. Research Impact. It is difficult to overstate the impact of the ACS-PRF award on my career and on the development of my research program at East Carolina University. Perhaps most importantly, this award has enabled me to fund stipends for undergraduate students over the past academic year and during the critical summer research period. It has also enabled me to recruit a talented graduate student with the promise of full research support over the upcoming academic year. As a result, I have been able to pursue multiple avenues of research while maintaining our department's mission of training the next generation of graduate students in the chemical sciences and future professional students in the health sciences. Furthermore, the awarding of this grant during my first year as a PI provided a significant boost to efforts to obtain future grant funding from other funding sources. Finally, having this particular project funded by the ACS-PRF is important for my career as it represents a new direction in research, distinct from research efforts as a doctoral student and post-doctoral scholar. As such, results obtained from this work will be critical for establishing my independence as an investigator.

II. Student Impact. Students who have been funded by the grant in Year 1 have been able to present their work in a variety of venues. At the departmental level, all of the students funded by this work have presented posters at the annual fall poster session sponsored by the department, in addition to presenting either talks or posters at the university-wide Research & Creative Activity Week. One undergraduate student has an upcoming poster presentation at SERMACS 2018 (Augusta, GA). Three undergraduate students who have participated in this project are currently applying to graduate school in the chemical sciences, professional programs in pharmacy, or are currently in dental school.

III. Research Funded by ACS-PRF and Results. The research plan put forward in the ACS-PRF award application consisted of five objectives, aimed at better defining the role of porcine pancreatic lipase (PPL) as a catalyst in several synthetically important carbon-carbon bond forming reactions in mixed organic solvents, with a particular emphasis on the role of the lid domain (red ribbon in **Fig. 1**), which plays an important role in interfacial activation of the lipase in organic solvents and substrate accessibility. The objectives were: I. Optimization of catalyst expression and purification; II. Investigation of promiscuous activities for three asymmetric C-C bond forming reactions; III. Investigation of multi-factorial conditions for favoring promiscuous catalysis; IV. Implementation of rational design strategies for enhanced catalytic activity and stereoselectivity; and V. Immobilization of catalysts and incorporation into hybrid systems. Much of our early efforts were focused on achieving Objective I, as expression and purification of a large quantity of PPL was necessary for completion of Objectives IV and V. We took a two-pronged approach to the expression of PPL, via the integration of a gene for PPL in the yeast *P. pastoris*, and via the creation of a plasmid for expression of PPL in *E. coli* (**Fig. 2**). While we were able to successfully integrate our lipase gene into multiple yeast strains, we were unable to achieve the high level of expression necessary for our proposed downstream experiments and applications. As a result, we are currently seeking out a collaborator with experience in large-scale expression of lipases in yeast. In addition, given the present expression challenges, and the ready availability of large quantities of lipases from a number of animal, plant, and fungal species, we expanded our objectives to include the screening synthetically important C-C bond forming reactions against a panel of commercially available lipases (**Table 1**). To date, we have screened a number of transformations against our lipase panel, and are currently evaluating

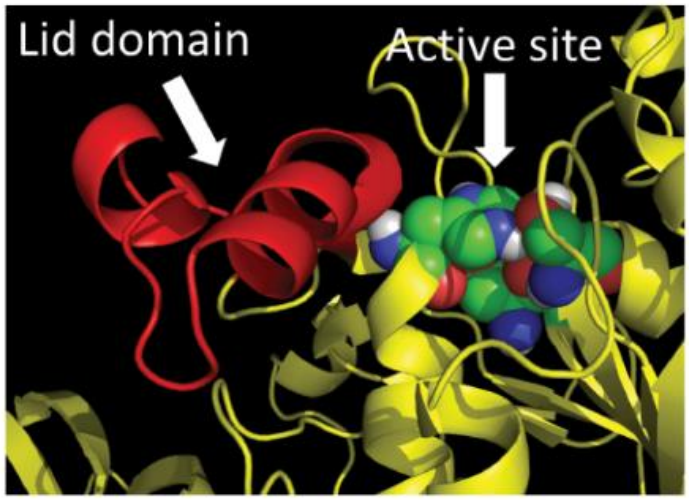

Figure 1. *Ribbon diagram generated from crystal structure of Porcine Pancreatic Lipase (PDB ID: 1ETH). Lid domain (red) is near the active site (colored spheres) and plays an important role in interfacial activation and substrate*

Table I. Lipase Panel
Rhizopus Olyzae
Wheat Germ
Candida rugosa
Aspergillus niger
Aspergillus oryzae
Pseudomonas cepacia
Candida sp.
Rhizopus niveus
Mucor miehei
Mucor javanicus
Candida antarctica "A"
Candida antarctica "B"
Pseudomonas fluorescens
Sus scrofa

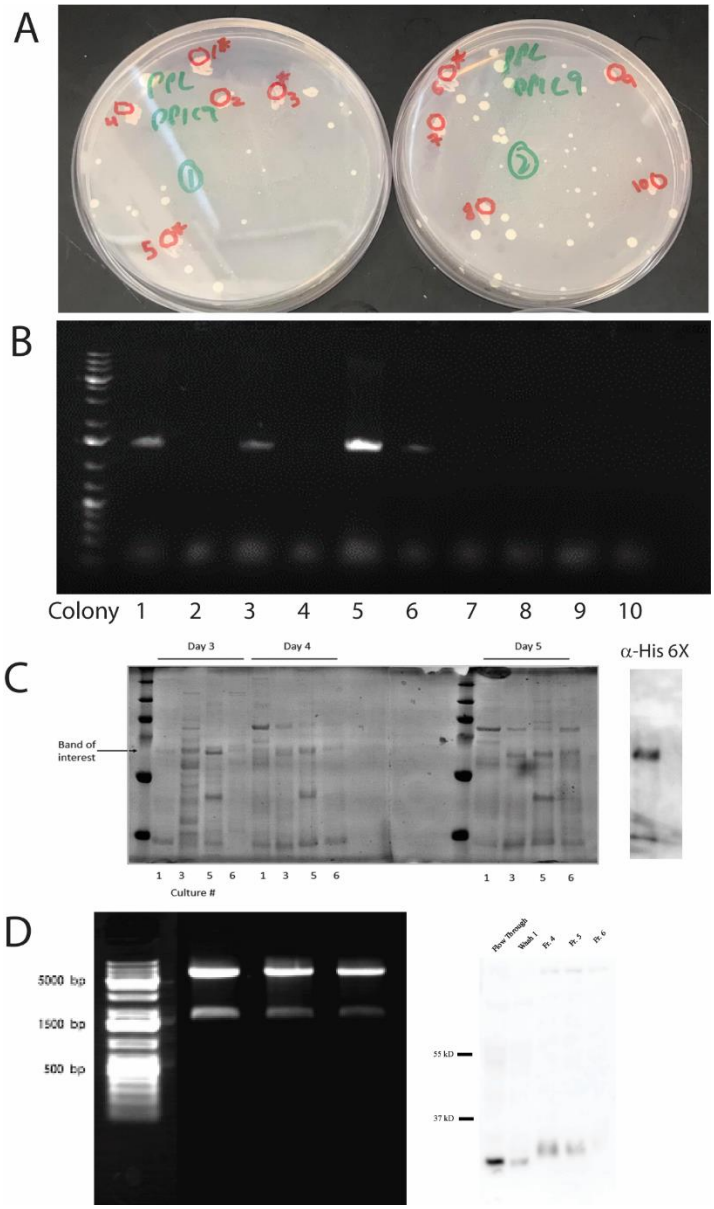

Figure 2. A. *Yeast transformants of plasmid pPIC9 in yeast strain KM71 and* ***B.*** *colony PCR for determination of successful PPL gene integration.* ***C.*** *Analysis of secreted expression in BMMY methanol induction media from pPIC9/KM71 cultures. α-6XHis western blot (right) of Day 4 culture from colony 5 confirms secreted expression of PPL-6XHis protein.* ***D****. HindIII/NdeI diagnostic digest of PPL inserts in pNIC28 expression vector (left). Bacterial expression (E. coli BL-21 (DE3); (right)) of His6X-PPL construct in pNIC-28 plasmid (α-6XHis western blot) yielded only truncated/degraded protein.*

these results. Our expanded research objectives have also resulted in two new collaborations: one with a mass spectrometry expert at ECU, Dr. Kimberly Kew, to develop a mass spec method based on literature precedent for the quantifying yield and enantioselectivity in a single analysis, and a second collaboration with ECU Associate Professor and crystallographer Dr. Jun-yong Choe to crystallize lipases with promiscuous catalytic activities in the presence of their non-native substrates. We believe that the resulting crystal structures will be of fundamental importance to the organo-biocatalysis community, either corroborating or challenging current assumptions regarding how these protein catalysts function in non-native transformations. In addition, we have also pursued preliminary work on Objective V (catalyst immobilization), where we were able to express and immobilize an enzyme on paramagnetic nanoparticles using a streptavidin-biotin protein capture strategy (**Fig. 3**). The proof-of-principle results obtained from this study demonstrate one possible path for lipase immobilization and application going forward.

IV. Conclusions While we have encountered some experimental challenges with the proposed work, these challenges motivated us to broaden our research plan to include two new, and potentially more fruitful, areas of investigation. In addition to the undergraduates who have invested significant time and effort into the initial phases of this project, the recent addition of a full-time graduate student is expected to accelerate progress on the proposed research.

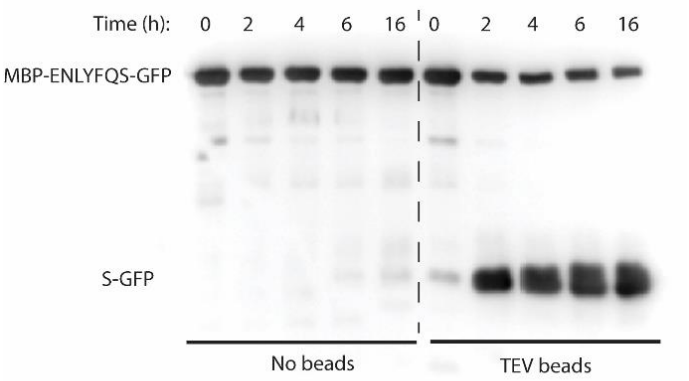

Figure 3. *Enzymatic activity of a TEV protease immobilized on paramagnetic nanobeads. Western blot (α-GFP) of TEV bead cleavage of a MBP-GFP protein fusion containing the ENLYFQS site (50 µL TEV beads; 200 µL of 0.1 mg/mL protein substrate). Blank (control) beads do not induce substrate proteolysis, while TEV beads induce efficient substrate cleavage after overnight incubation at 30 °C (left panel).*