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. The ACS PRF award has enabled the pursuit of numerous avenues of research, including screening for new biocatalytic transformations and the development of methods for biocatalytic enzyme immobilization. Currently, we are preparing two manuscripts based on our work screening lipases for catalysis of new C-C bond forming reactions and one manuscript based on our enzyme immobilization work. In addition, one collaborative proposal has been submitted based on the enzyme immobilization work (with ECU Brody School of Medicine faculty member Jitka Virag).

II. Student Impact. Students who have been funded by the grant in Year 2 have been able to present their work in a variety of venues. One graduate student presented work funded by ACS PRF at the spring national meeting of the American Chemical Society in Orlando, FL (Screening and characterization of C-C bond forming reactions catalyzed by promiscuous biocatalysts in non-aqueous media. Mitul Patel*, Nathaneal Green*, Jacob Burch*, Kimberly Kew, Robert Hughes), and two upcoming student presentations are scheduled for SERMACS 2019 (Identification and characterization of biocatalysts for synthesis of the Wieland-Miescher ketone. Mitul Patel*, Robert Hughes; Screening and characterization of a commercially available lipase library for catalysis of a Morita-Baylis-Hillman reaction. Karla D. Hernandez Gomora**, Robert M. Hughes). Karla D. Hernandez Gomora was an NSF REU summer student whose work was funded by both the ACS PRF and the NSF REU. Enzyme immobilization work featuring contributions from undergraduates was also presented at the spring ACs meeting (Immobilization of enzyme fusions on superparamagnetic nanoparticles: Two case studies. Jessica Norris*, William Taylor*, Robert Hughes). 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. This grant is currently providing research stipends for two MS students. (* ECU student; ** UNC-Charlotte student)

III. Research Funded by ACS-PRF and Results. Our current ACS-PRF projects are focused on the screening synthetically important C-C bond forming reactions against a panel of commercially available lipases. While it is fairly well known that lipase preparations are rather crude mixtures, potentially containing dozens of catalytically active species, relatively few efforts have focused on identifying which proteins are in fact responsible for the "promiscuous catalysis" observed with lipases in non-aqueous solvents [1]. This is a critical bottleneck for the field of biocatalysis, as follow up efforts to improve novel protein biocatalysts (via directed evolution or rational design) are impossible when the identity of the true catalyst remains in question. In our lab, we have taken a biochemist's approach to promiscuous catalysis, using proteomics to identify the components of biocatalytic lipase preparations in an attempt to better understand how these catalysts actually work. To date, we have characterized three lipase preparations and identified the most abundant protein species in the mixtures. These efforts have revealed some rather surprising results that we intend to publish soon. In addition, we have screened a commercially available lipase library against three model C-C bond forming transformations: a Robinson Annulation, a Knoevenagel Condensation, and a Morita-Baylis-Hillman reaction. Of particular interest in this work has been a follow-up study to the synthesis of Weiland-Meischer ketone from methyl vinyl ketone and 2-methyl-1,3-cyclohexanedione. Classic biocatalytic syntheses of this molecule using L-proline and the catalytic antibody 38C2 have provided an invaluable point of departure for investigating lipase-catalysis of this molecule [2, 3]. While it is unlikely that we will improve on the yields obtained with previously reported biocatalysts, lipase catalysis has the potential to provide enhanced stereoselectivity and the advantage of significant cost savings over catalytic antibody production. Recently, we found that inclusion of an amine co-catalyst is necessary for efficient lipase-mediated biocatalysis of this transformation [4].

IV. Conclusions The work funded by the ACS-PRF grant has yielded new directions for our laboratory and has supported numerous undergraduate and graduate student stipends and conference travel. We are currently focused on getting our initial studies into the scientific literature and utilizing the same results as the basis for upcoming grant applications.

REFERENCES

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