



**BIOMEDICAL
SCIENCES**
PhD PROGRAM

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DISSERTATION DEFENSE

ANGELA CAMPO
PhD Candidate

**“NMR Metabolomics for Cell-Free Protein Synthesis
Optimization”**

Friday, April 23rd, 2021

1:00 p.m.

Cisco Webex:

<https://wright.webex.com/wright/j.php?MTID=m6ac22b99616a4fab5a807000785e66bc>

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Campo, Angela, Biomedical Sciences PhD Program Wright State University, 2021

Cell-Free Protein Synthesis (CFPS) has been utilized by biochemists to produce a variety of chemicals and therapeutics. While CFPS has spawned research in the biochemistry and medical communities, there are still unknown issues with interlaboratory variability with the technique. This work explored the black box nature of CFPS reactions by analyzing the CFPS reactions *in situ* with Nuclear Magnetic Resonance (NMR) spectroscopy. Aim 1 developed the protocol for conducting NMR experiments on *E. coli* cell-free reactions as well as a data analysis pipeline. This was accomplished with ^1H NMR, capturing metabolite changes over time. The 1D NOESY experiment proved to provide good signal-to-noise for consecutive 9-minute acquisitions. Aim 2 explored the differences between lysates produced by different laboratories as well as how they behave in reactions. The proton data indicated there are detectable differences between the lysates as well as how they perform in reactions. Ethanol production in lysates and in reactions was a major difference between lysates. In Aim 3, ^{13}C -enriched phosphoenolpyruvate was utilized as a tracer to determine which metabolic pathways were activated during cell-free reactions. The ^{13}C NMR data indicated glycolysis was activated as expected but also that gluconeogenesis was occurring. Ethanol production was also detected, but from more than one carbon source. Insight such as this can guide future genetic modifications to the chassis organism and identifying side products that may be wasting resources and decreasing product yields.