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Towards Discovering Inhibitors of cyt c Biosynthesis in Systems I, II, and III

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Cytochrome c (cyt c) is a heme protein found in most organisms (including human pathogens) that plays an essential role in both aerobic and anaerobic growth. The biogenesis of c-type cytochromes occurs by three different systems (Systems I and II in bacteria and System III in humans). Besides requiring different protein systems, their site of synthesis is also different. Systems I and II function in the periplasmic space while System III functions in the mitochondrial inter-membrane space. These differences may allow for selective targeting of bacterial systems using antimicrobial compounds which may be beneficial in combating infectious bacterial diseases. My goal is to develop a robust assay that detects cyt c maturation quantitatively to allow for subsequent analyses of levels of cyt c maturation in the presence and absence of potential inhibitors. In recombinant E. coli, Systems I and II produce cyt c in the periplasm while System III makes cyt c in the cytoplasm. An *in vivo* assay has been developed in the Kranz lab that detects cyt c produced in the periplasm, but cannot detect production in the cytoplasm. Therefore, I have been optimizing an *in vitro* method to extract cyt c from recombinant E. coli grown in small volumes (<5mL) and detect it quantitatively by heme stain. Here I present work showing that the *in vitro* assay can detect differences in the amount of cyt c produced within System III. I also explored the optimal conditions for comparing cyt c biogenesis across the three systems in recombinant E. coli. Future direction is to test known inhibitors of heme biosynthesis and protein biosynthesis to test the effectiveness of this assay.