

438j Towards the Commercial Production of Pharmaceutical Proteins Using Cell-Free Systems

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Energy substrates and nucleotides represent the major reagent costs associated with traditional cell-free protein synthesis systems. In order for cell-free technology to effectively compete with conventional *in vivo* rDNA protein expression, these costs must be reduced. Two recent breakthroughs have demonstrated that (1) the most efficient arm of cellular energy production, oxidative phosphorylation, has been activated to solve the ATP supply issue *in vitro*, and (2) cell-free protein synthesis reactions can be fueled by exchanging nucleoside triphosphates (NTPs) with nucleoside monophosphates (NMPs) when 10mM phosphate is supplemented in cell-free reactions utilizing glucose metabolism. Here, we demonstrate the effects of combining these two breakthroughs on the overall system performance. We obtain protein yields of 700 ug/mL of chloramphenicol acetyl transferase (CAT) in a 5-hour batch reaction, and we characterize the activated cell-free metabolism with HPLC analysis. These results increase the attractiveness of cell-free protein biosynthesis as a commercial expression technology.