

P018 Kinetics and mechanism of CB1954 reduction by *E. coli* nitroreductase

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E. coli NfsB is a flavoprotein with broad substrate specificity, reducing nitroaromatics and quinones; using either NADPH or NADH. One of its substrates is the prodrug CB1954, which is converted to a cytotoxic agent; so NfsB/CB1954 has potential for use in cancer gene therapy; however wild-type NfsB has poor kinetics of CB1954 reduction. Site-directed mutagenesis and screening in vivo has produced several NfsB mutants with selectively improved specificity constants for CB1954. We have compared the steady-state and stopped-flow kinetics of the most promising single, double, and triple mutants with wild-type enzyme, using several substrates. Additionally, computational methods have been utilized to study potential underlying reaction mechanisms so as to identify the order of electron and proton transfers that make up the initial reduction step and the sources of the protons. We have also used molecular dynamics to examine the nature of the active site of the wild-type enzyme, mutants, and other nitroreductases, in order to both understand the current processes, as well as to design new mutants with greater efficacy.