Supplemental Data

Structural Insights into Intermediate Steps

in the Sir2 Deacetylation Reaction

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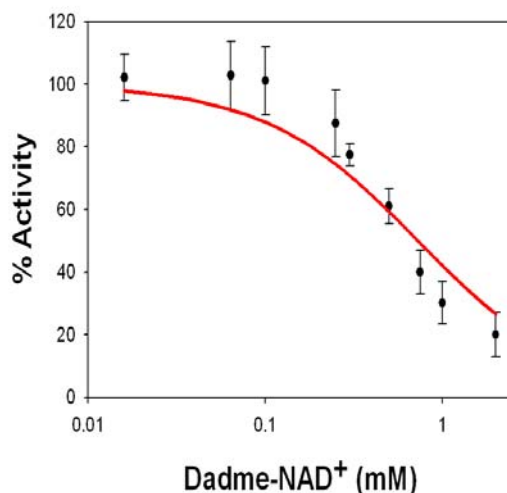Vern L. Schramm, Weiping Zheng, and Cynthia Wolberger**

Figure S1. DADMe-NAD^{+} Inhibition of Sir2Tm Activity
DADMe-NAD^{+} inhibition reactions were preformed with 50 ug/ml of Sir2Tm wild-type protein were
incubated at 37°C for 15 minutes with 125 uM of NAD^{+}, 500 uM of acetyl P53 peptide, and various
concentrations of DADMe-NAD^{+}. Data were fit with an equation 1 to determine the IC_{50} value for
DADMe-NAD^{+} in SigmaPlot:

$$V_I = V_o(1-[I / (IC_{50} + I)]),$$

where v_o is the initial rate of uninhibited reaction and v_I is the initial rate of reaction in the presence of
inhibitor. The determined IC50 for DADMe-NAD+ was 720 uM. The K_i value was determined by
substituting the IC_{50} value for DADMe-NAD^{+} and K_M value for NAD^{+} (125 uM) into:

$$K_i = IC_{50} / (1 + ([NAD^+] / K_M)),$$

where [NAD^{+}] is the concentration of NAD^{+} used in the assay. The determined K_i value for DADMe-
NAD^{+} is 360 uM.