SUPPORTING INFORMATION

Probing the Role of Active Site Residues in NikD, an Unusual Amino Acid Oxidase that Catalyzes an Aromatization Reaction Important in Nikkomycin Biosynthesis†

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Running Title: Probing the Role of Active Site Residues in NikD Catalysis

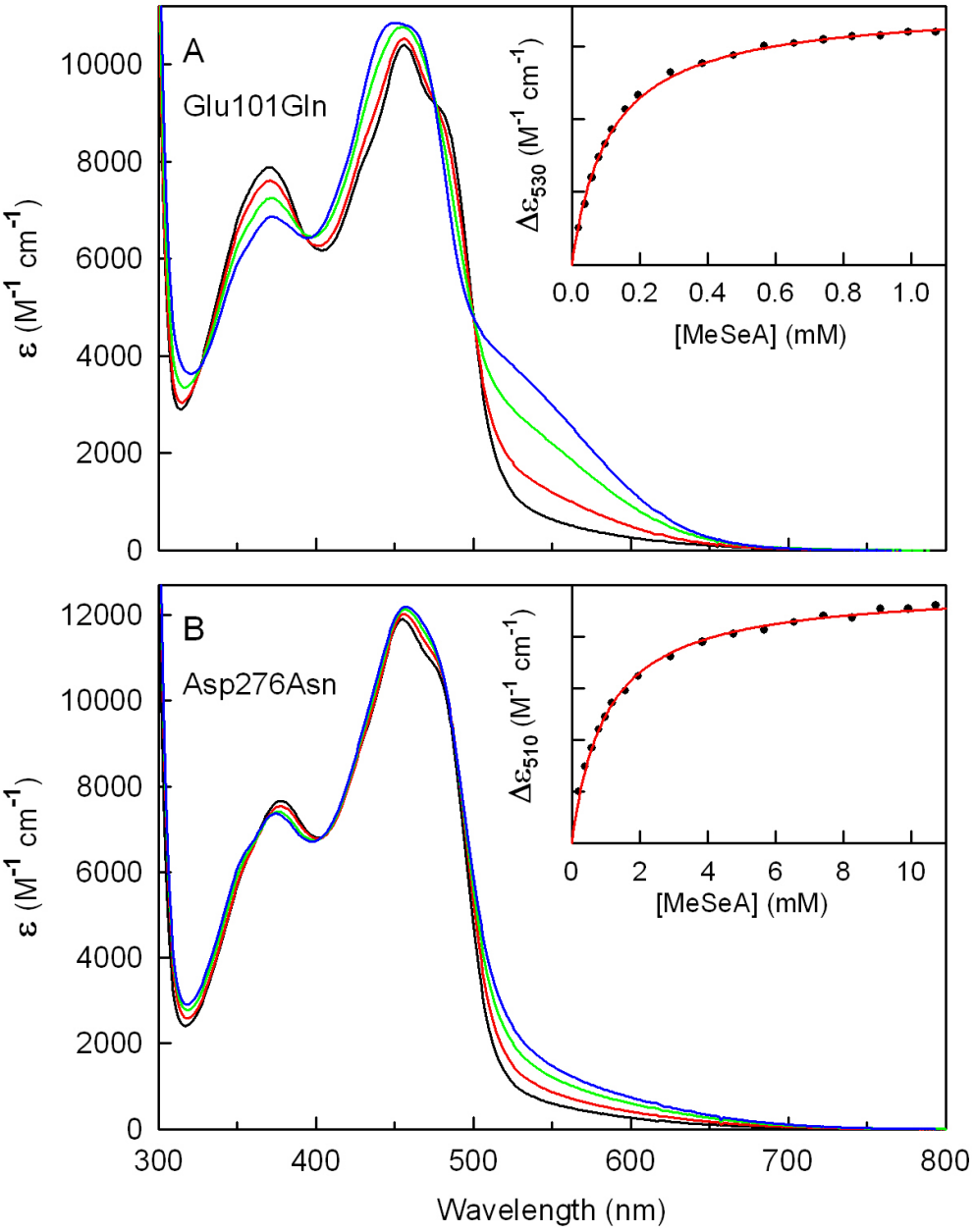

Figure S1 Spectral properties of complexes formed with Glu101Gln or Asp276Asn and methylselenoacetate (MeSeA). Spectral titrations were conducted in 100 mM potassium phosphate buffer pH 8.0 at 25 ºC. The blue curve in each panel is the absorption spectrum calculated for 100% complex formation, as described in Experimental Procedures. Panel A: Absorption spectra of Glu101Gln in the presence of 0, 0.04 and 0.2 mM MeSeA are shown in the black, red and green curves, respectively. Panel B: Absorption spectra of Asp276Asn in the presence of 0, 0.6 and 2.91 mM MeSeA are shown in the black, red and green curves, respectively. The inset in panel A or B shows a plot of the change in extinction at 530 or 510 nm, respectively, as a function of the concentration of MeSeA. The solid red lines were obtained by fitting a theoretical binding curve ($\Delta\epsilon_{obs} = \Delta\epsilon_{max}[ligand]/(K_d + [ligand])$ to the data.

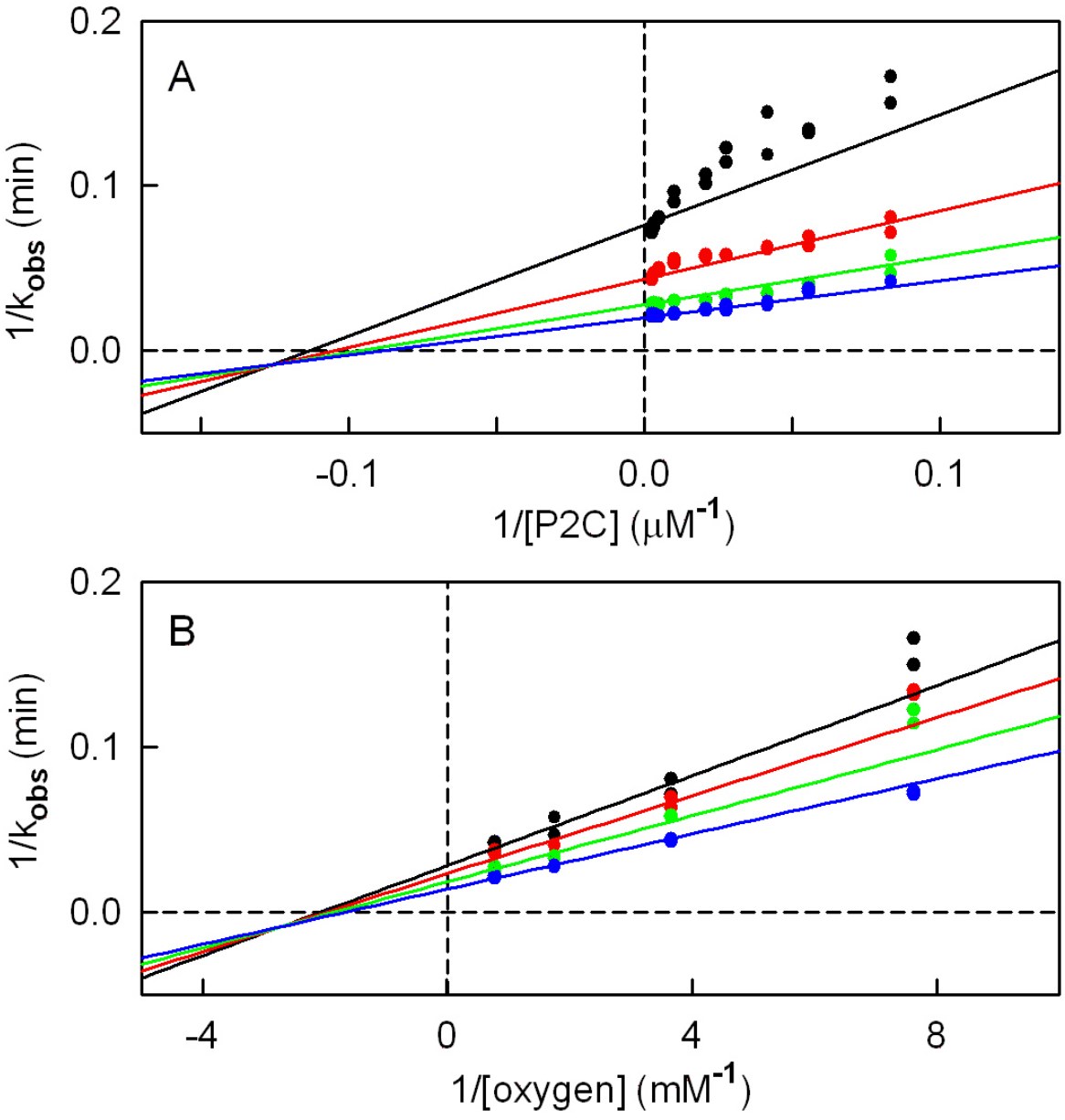

Figure S2 Steady-state kinetic analysis of P2C oxidation by Asp276Asn. Reactions were conducted by monitoring picolinate formation at 264 nm in 100 mM potassium phosphate buffer, pH 8.0, at 25 ºC. The solid lines in panels A and B were obtained by fitting equation 1 to the data. Panel A: The black, red, green, and blue circles show data obtained at 0.13, 0.27, 0.57, and 1.29 mM oxygen, respectively. Panel B: For clarity, data at selected P2C concentrations (12.0, 18.0, 36.0, and 400 μM) are shown by the black, red, green, and blue circles, respectively.

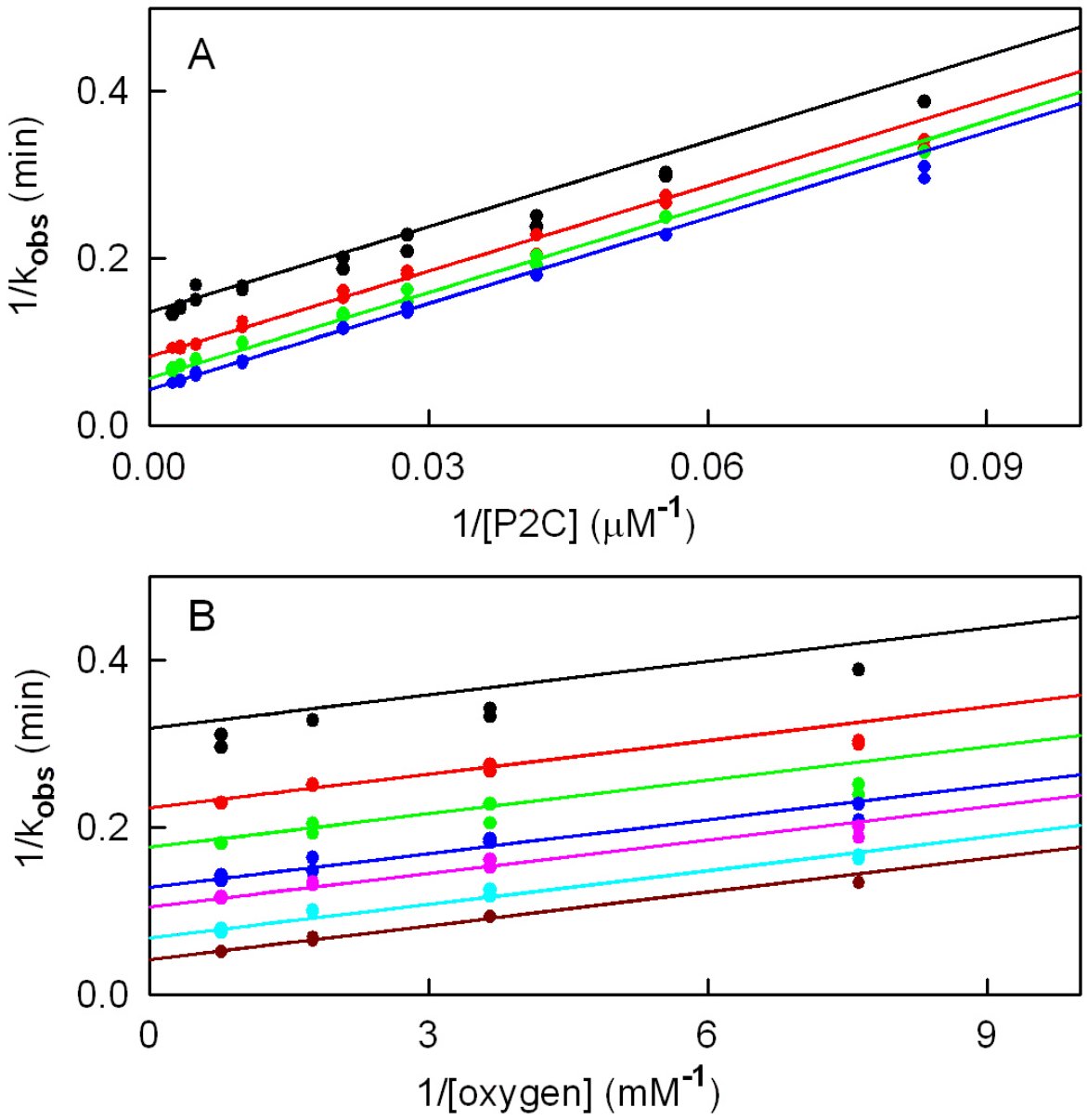

Figure S3 Steady-state kinetic analysis of P2C oxidation by Glu101Gln. Reactions were conducted by monitoring picolinate formation at 264 nm in 100 mM potassium phosphate buffer, pH 8.0, at 25 ºC. The solid lines in panels A and B were obtained by fitting equation 2 to the data. Panel A: The black, red, green, and blue circles show data obtained at 0.13, 0.27, 0.57, and 1.29 mM oxygen, respectively. Panel B: For clarity, data at selected P2C concentrations (12.0, 18.0, 24.0, 36.0, 48.0, 100, and 400 μM) are shown by the black, red, green, blue, magenta, cyan, and brown circles, respectively.