

Supplemental Material:

Figure S1: pH dependence of H228A and H11A SsuD activity. Reactions were initiated by the addition of NADPH (500 μM) into a reaction mixture containing H228A SsuD (0.2 μM), SsuE (0.6 μM), FMN (2 μM), and a range of 1-octanesulfonic acid concentrations (10–5000 μM) in either 50 mM Bis-Tris (pH range of 5.8–7.2), 50 mM Tris-HCl (pH range of 7.2–9.0), or 50 mM glycine (pH range of 9.0–10.0) and 100 mM sodium chloride at 25°C. pH dependence of H228A SsuD (●) A: k_{cat} values B: $k_{\text{cat}}/K_{\text{m}}$ values; pH dependence of H11A SsuD (▲) C: k_{cat} values D: $k_{\text{cat}}/K_{\text{m}}$ values. Each point is the average of at least three separate experiments. Solid lines for A and C are fits of the data to eq. 3:

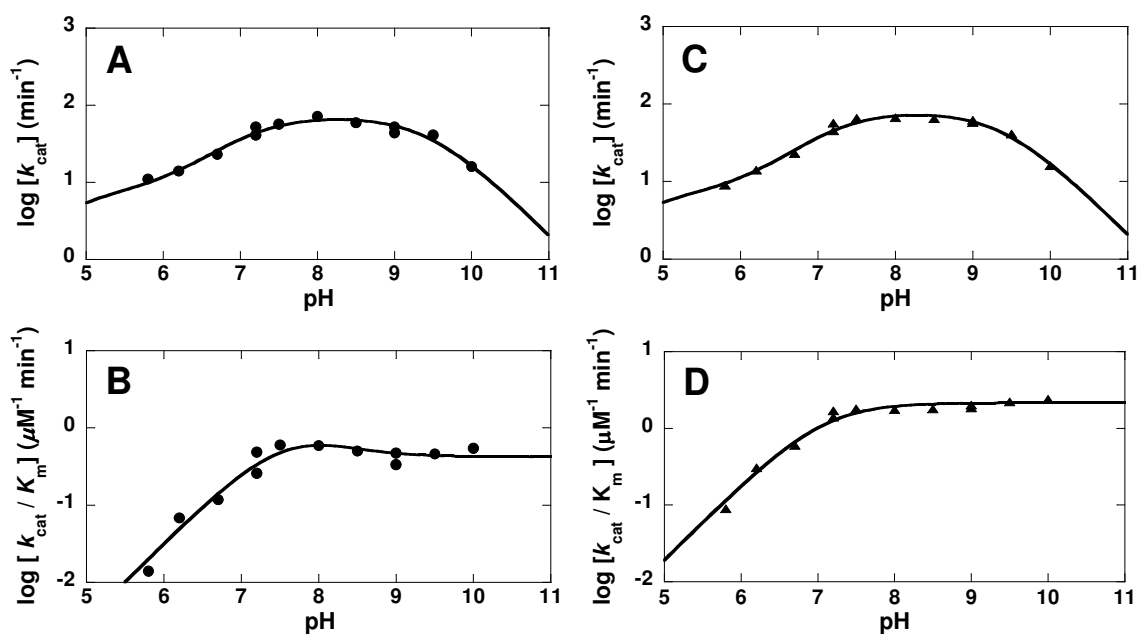


Figure S2: Emission intensity measurements at 344 nm were measured using an excitation wavelength at 280 nm. The change in the emission intensity at 344 nm following each addition of FMNH₂ was converted to the estimated concentration of bound FMNH₂ to SsuD (eq 4) and plotted against the concentration of free FMNH₂. A: The titration of R226A SsuD enzyme (0.5 μ M) with FMNH₂ (0.26-8.26 μ M). B: The titration of R226K SsuD enzyme (0.5 μ M) with FMNH₂ (0.26-8.26 μ M). The solid line in each plot represents the fit of the titration curve to eq 5. Each titration was performed in triplicate.

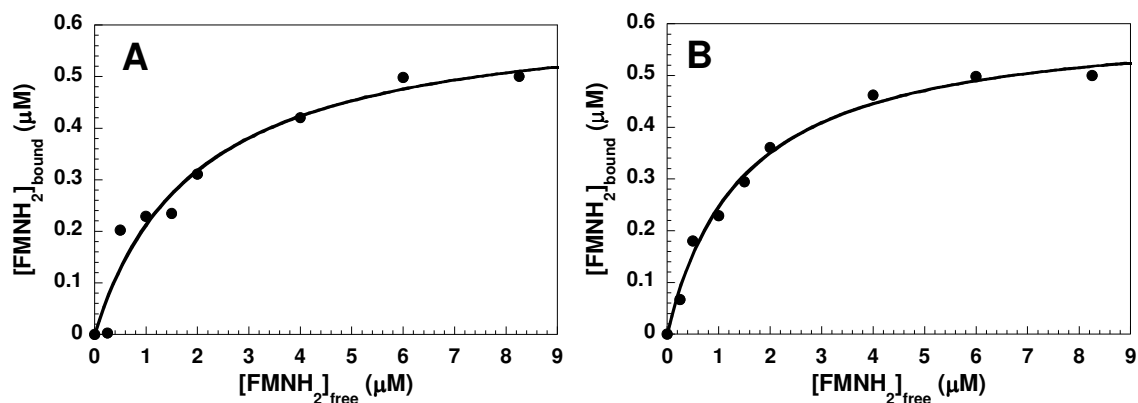


Figure S3: Emission intensity measurements at 344 nm were measured using an excitation wavelength at 280 nm. The change in the emission intensity at 344 nm following each addition of 1-octanesulfonate was converted to the estimated concentration of bound 1-octanesulfonate to SsuD-FMNH₂ enzyme complex (eq 4) and plotted against the concentration of free octanesulfonate. A: The titration of R226A SsuD-FMNH₂ enzyme complex. R226A SsuD (1 μ M) was premixed with FMNH₂ (2 μ M) and titrated with 1-octanesulfonate (0.25-108 μ M). B: The titration of R226K SsuD-FMNH₂ enzyme complex. R226K SsuD (1 μ M) was premixed with FMNH₂ (2 μ M) and titrated with 1-octanesulfonate (0.25-108 μ M). The solid line in each plot represents the fit of the titration curve to eq 5. Each titration was performed in triplicate.

