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**Supporting information for article:**

# Structure of the *Bacillus anthracis* dTDP-L-rhamnose biosynthetic enzyme glucose-1-phosphate thymidylyltransferase (RfbA)

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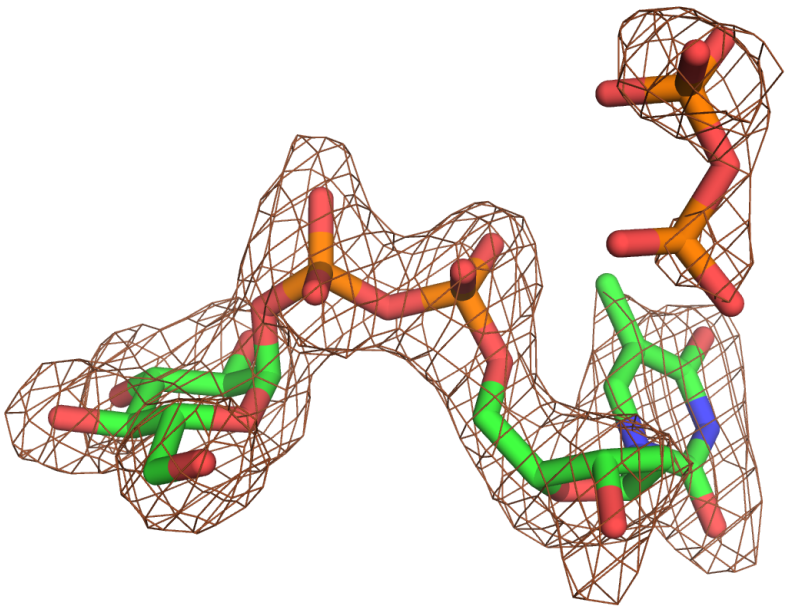

**Figure S1.** Fo-Fc electron density map for RfbA 4ECM structure. The Fo-Fc electron density omit map (brown mesh) for dTDP-α-D-glucose (green sticks) and $PP_i$ (orange sticks) is contoured at 3σ. The map was calculated after removing the ligands from the final model.

**Table S1.** Kinetic parameters for RfbA enzyme toward different nucleotides.

| Substrate | $V_{max}$ (μmol/min/mg) | $S_{0.5}$ (μM) | n | $k_{cat}/S_{0.5}$ ($M^{-1}s^{-1}$) |
|---|---|---|---|---|
| dTTP | 6.60 ± 0.10 | 70 ± 4 | 1.26 ± 0.08 | $4.31 \times 10^4$ |
| UTP | 11.10 ± 0.36 | 220 ± 19 | 1.90 ± 0.26 | $2.31 \times 10^4$ |
| Glc1P (with dTTP) | 4.30 ± 0.08 | 30 ± 2 | 1.91 ± 0.14 | $6.57 \times 10^4$ |
| Glc1P (with UTP) | 13.30 ± 0.67 | 270 ± 13 | 1.06 ± 0.10 | $2.26 \times 10^4$ |
| $Mg^{2+}$ (with dTTP) | 8.80 ± 0.13 | 550 ± 15 | 3.77 ± 0.26 | $7.33 \times 10^3$ |
| $Mg^{2+}$ (with UTP) | 13.30 ± 0.04 | 330 ± 2 | 2.30 ± 0.03 | $1.85 \times 10^4$ |

*Enzyme Assays*—The kinetic activity of RfbA was tested using a previously described Malachite Green assay (Fusari *et al.,* 2006). RfbA enzyme activity toward a variety of nucleotides was first determined in the presence of 50 mM HEPES pH 8.0, 4 mM $MgCl_2$, varying concentrations (0-2mM) of nucleotides (either dTTP, UTP, CTP, ATP, or GTP), 0.5 mM glucose 1-phosphate, 1.5 U/mL pyrophosphatase, and 0.2 mg/mL bovine serum albumin for 10 min at 310.15K in the presence of 83 nM enzyme in triplicate. RfbA activity was only observed in the presence of either dTTP or UTP. Kinetic parameters were then determined for dTTP, UTP, Glc1P, and $Mg^{2+}$ by varying concentrations of the substrate/cofactor of interest (0-12 mM $MgCl_2$; 0-2 mM dTTP, UTP, or Glc1P) and then fitting data to a modified Hill equation as described previously (Fusari *et al.,* 2006).

*Kinetic parameters for RfbA*—We found the RfbA protein was only active toward dTTP and UTP, not CTP, ATP, or GTP, with the catalytic efficiency being approximately two-fold higher toward dTTP than UTP. Additionally, we observed substrate inhibition by Glc1P at concentrations greater than 500μM in the presence of dTTP (1mM). This inhibition was not observed when UTP was used. Data points prior to inhibition were used to calculate the kinetic parameters for Glc1P in the presence of dTTP. Further exploration into the kinetic behavior of this enzyme is necessary to determine the rationale for this substrate inhibition.

Reference:

Fusari, C., Demonte, A.M., Figueroa, C.M., Aleanzi, M., Iglesias, A.A. (2006) *Anal. Biochem.* **352**, 145-147.