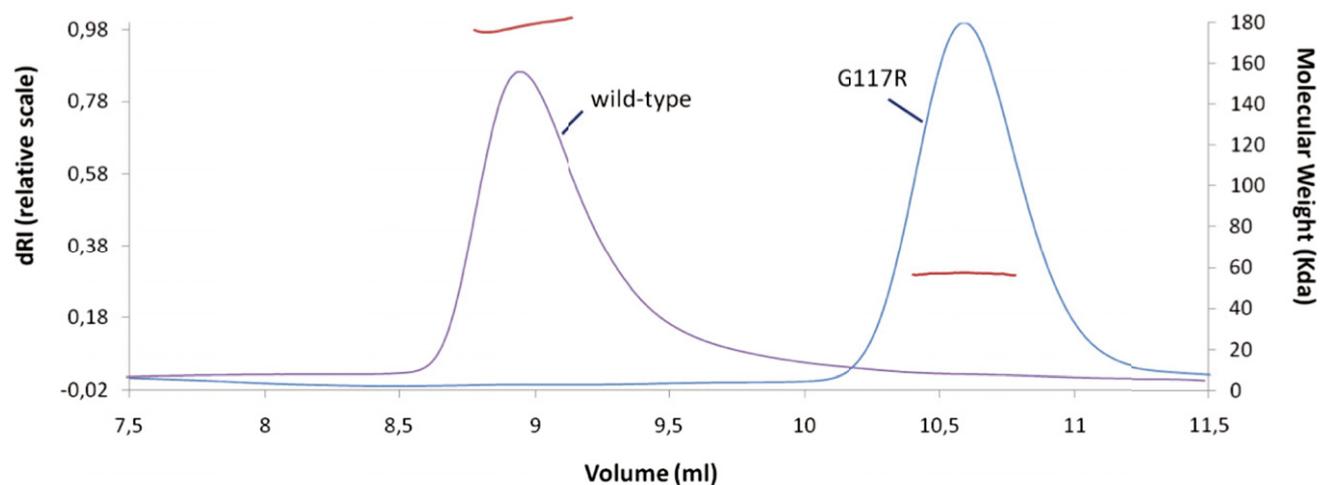


Supporting Information

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Construct	Calculated MW	Expected MW	Oligomeric State
Wild-type	168.7 kDa	57.3 kDa	2.95 (Trimer)
G117R	56.8 kDa		0.99 (Monomer)

Fig. S1. SEC-MALS elution profiles of WT TarM and its trimerization interface mutant, G117R. Both proteins were run at a concentration of 25 μ M. Horizontal lines correspond to calculated molecular weights as listed.

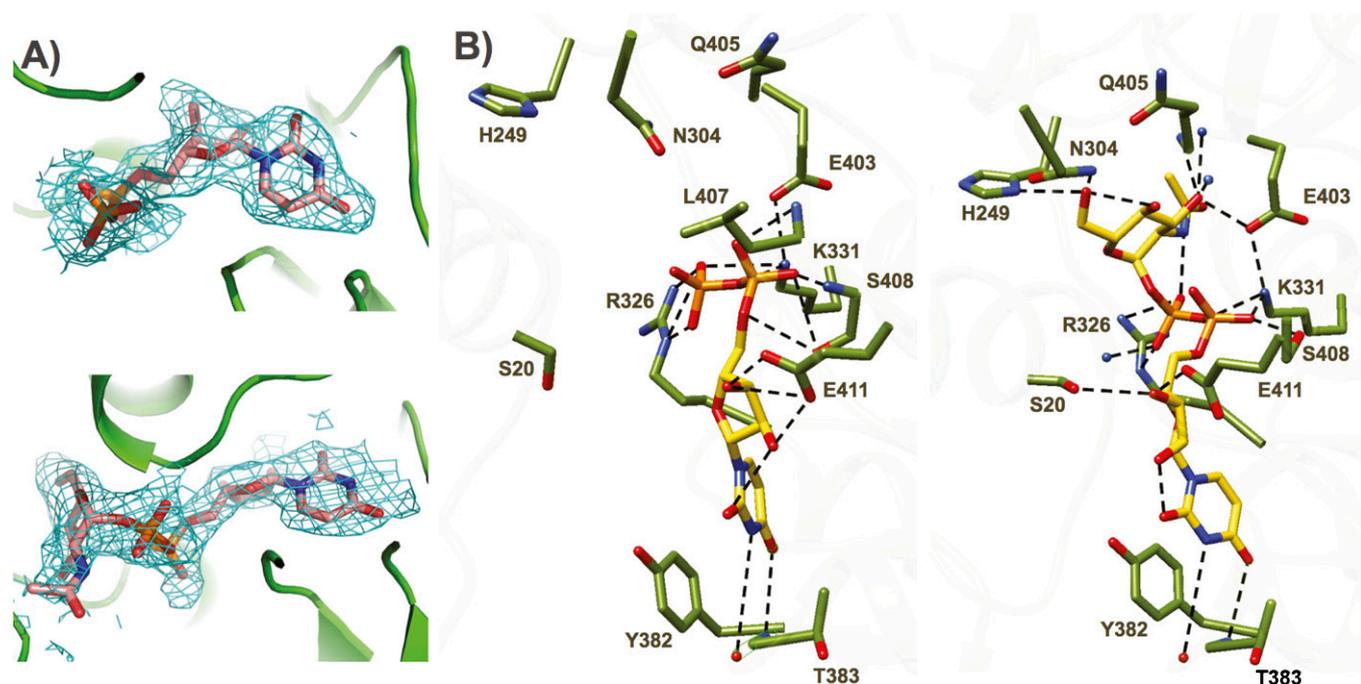


Fig. S2. TarM monomer catalytic site in complex with donor-substrate. (A) Ligand mFo-DFc OMIT maps for UDP (Top) and UDP-GlcNAc (Bottom) contoured at 3σ . (B) Interaction of active site residues with cleaved UDP remnant (Left) and intact UDP-GlcNAc (Right). Substrates and relevant residues are displayed in stick form and colored according to heteroatom type, with carbons of enzyme residues displayed in green and those of UDP/UDP-GlcNAc in yellow. Polar interactions between atoms are displayed by dotted lines. Backbone carbonyls and amides are displayed as red and blue spheres, respectively, where applicable.

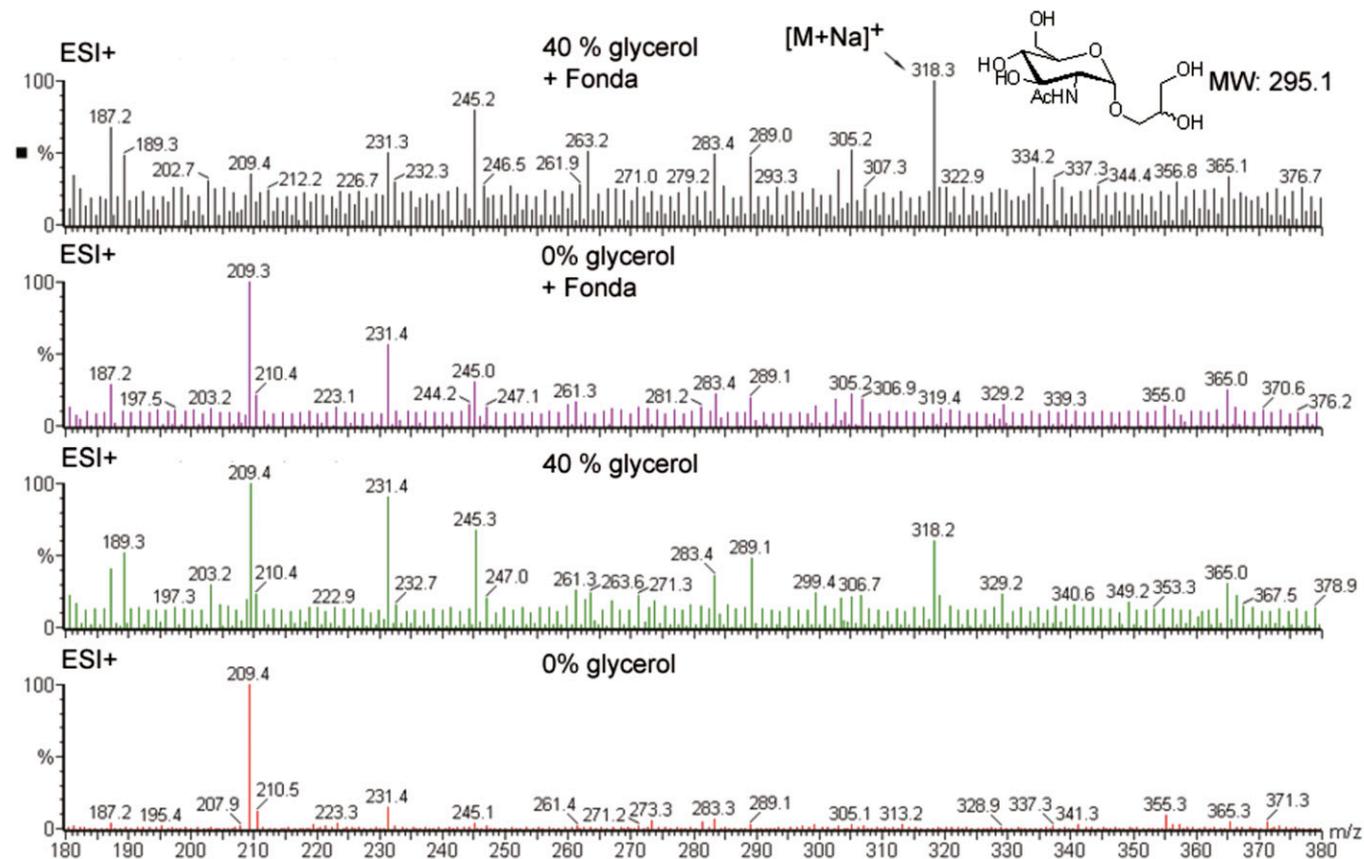


Fig. S3. The products of TarM reaction mixtures containing 10 mM UDP-GlcNAc \pm 1 mM fondaparinux (Fonda) and \pm 40% glycerol where indicated were diluted 1:40 (vol/vol) with MeOH and analyzed via flow injection analysis and detected by electrospray ionization MS (positive mode).

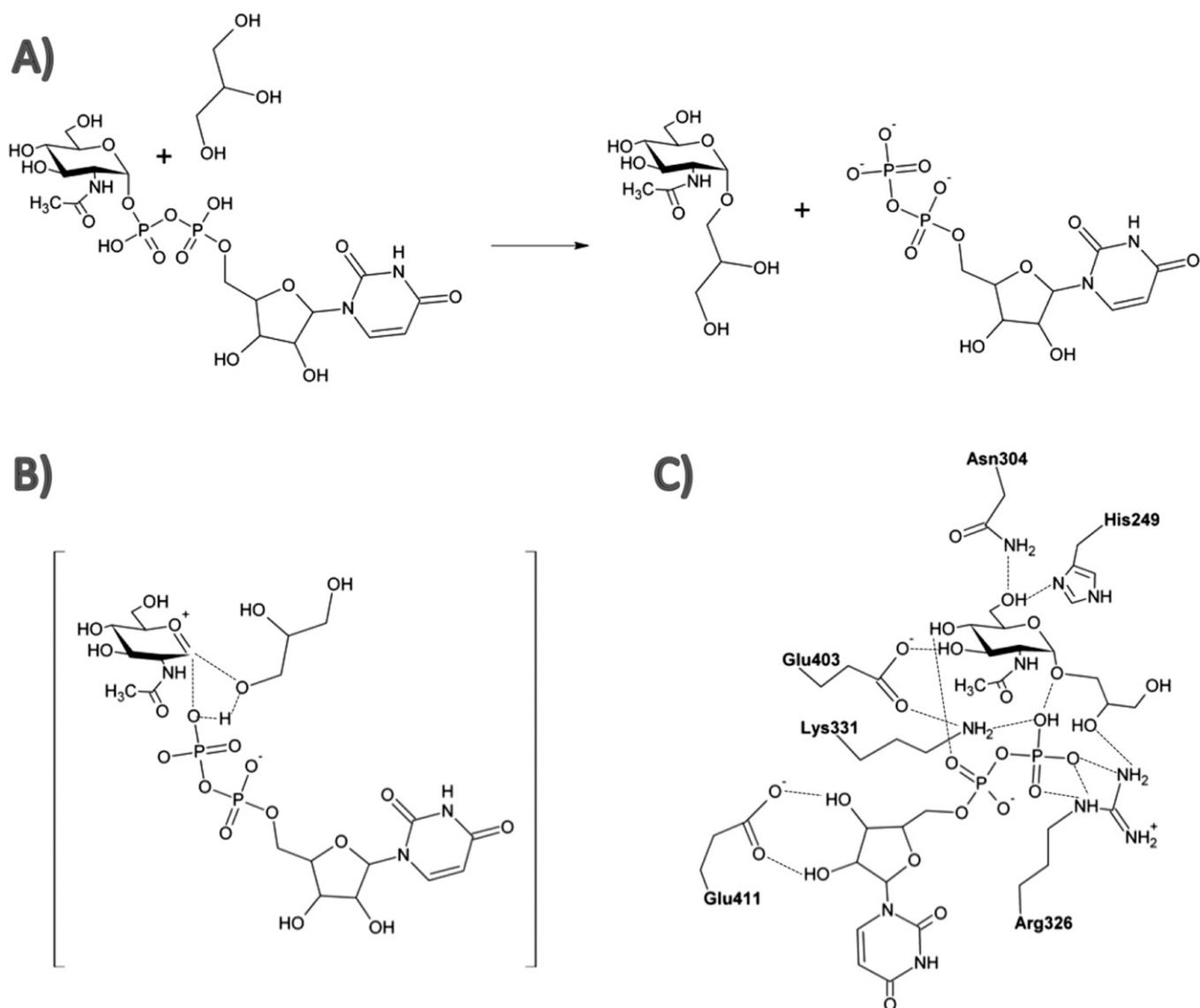


Fig. S8. Schematic of the proposed TarM reaction mechanism. (A) GT reaction between UDP-GlcNAc and glycerol resulting in UDP and α -glyceryl-GlcNAc products. (B) Proposed front-face oxocarbenium ion-like transition state preceding product formation. (C) TarM ternary complex with bound UDP and α -glyceryl-GlcNAc. Polar interactions between atoms are displayed by dotted lines.

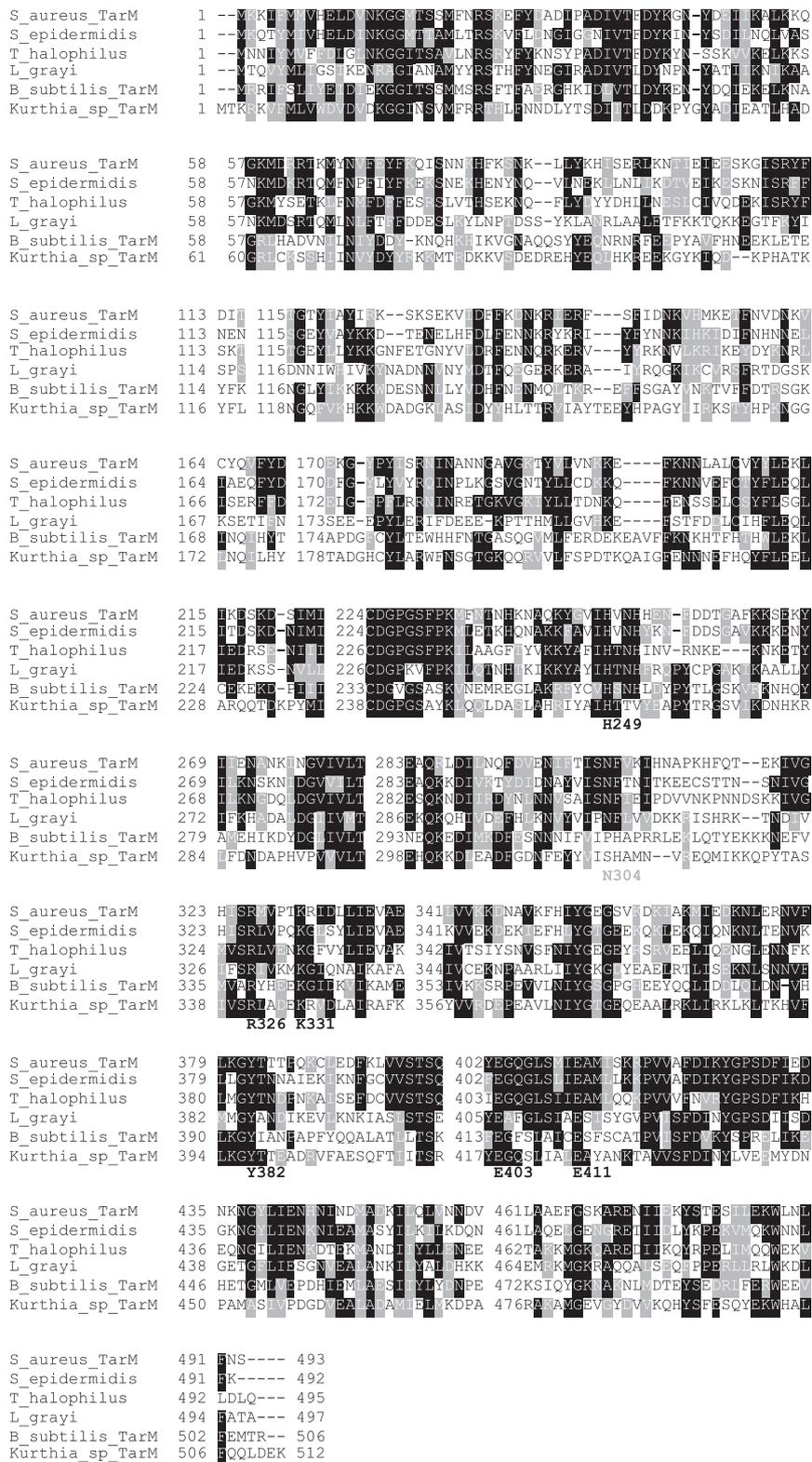


Fig. S9. Sequence alignment of *Staphylococcus aureus* TarM with other bacterial species and genera. *S. aureus* TarM (National Center for Biotechnology Information reference sequence YP_185908.1) was aligned with sequences from *Staphylococcus epidermidis* (WP_002502944.1), *Tetragenococcus halophilus* (YP_004887116.1), *Listeria grayi* (WP_003756742.1), *Bacillus subtilis* (WP_019259626.1), and *Kurthia* sp. (WP_010286982.1). Similar sequences were identified with BLAST by using the nonredundant protein sequence database. Sequence alignment was performed with ClustalW. Letters blocked in black indicate complete identity, and those blocked in gray indicate similarity to the column consensus.

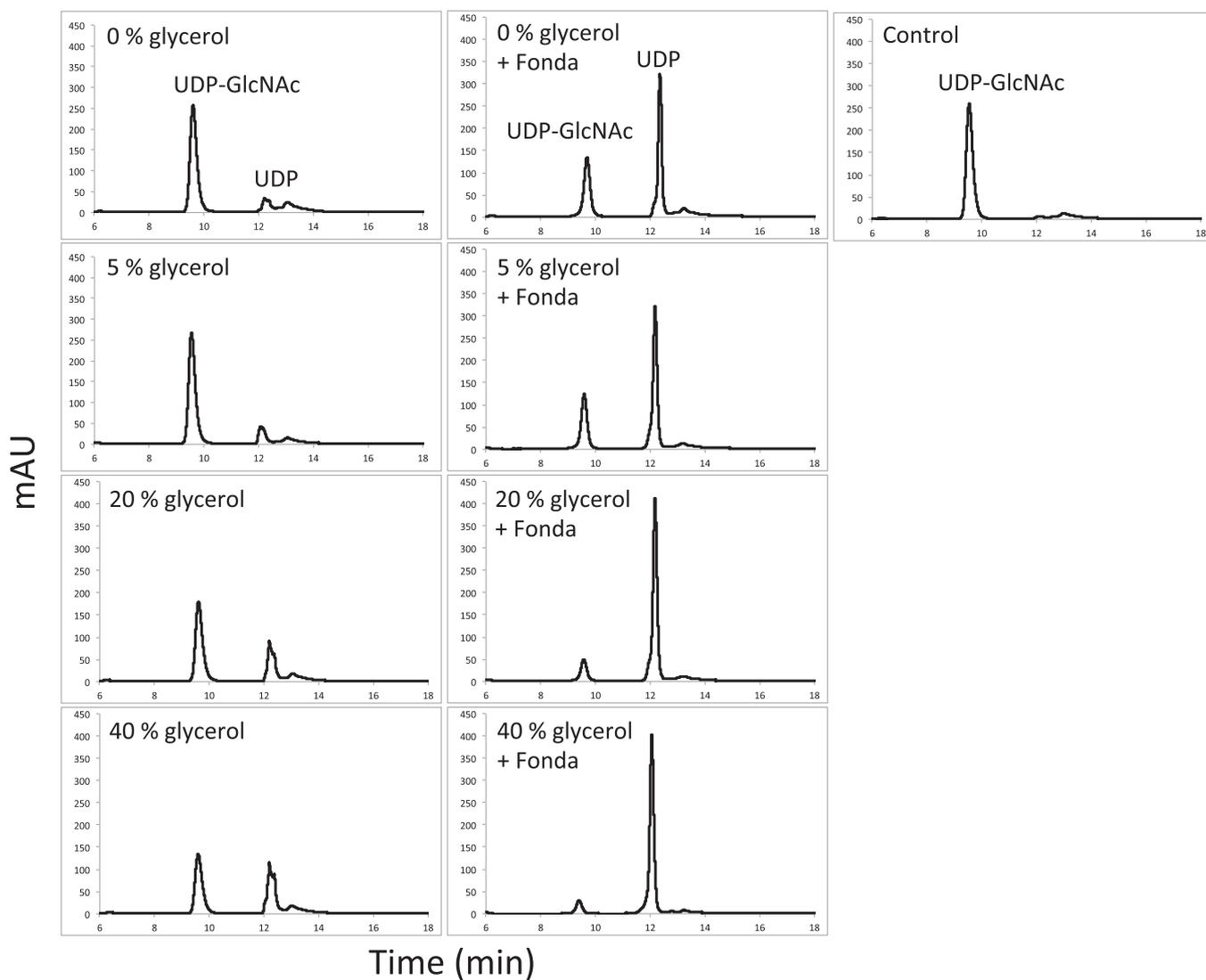


Fig. S10. HPLC chromatogram of UDP-GlcNAc hydrolysis with increasing concentrations of glycerol. UDP-GlcNAc was incubated with WT TarM in the presence of increasing glycerol and 1 mM fondaparinux (+ Fonda) as indicated for 1 h. UDP-GlcNAc and the hydrolyzed UDP were separated on a DEAE weak anion exchange column and the peak signals detected at 254 nm. UDP-GlcNAc in the absence of TarM is shown as a control.



Movie S1. Structural morph between TarM open and closed states. Open state from TarM:UDP complex and closed state from TarM:UDP-GlcNAc complex (found in asymmetric unit of TarM G117R structure). Fondaparinux (yellow), glyceryl-GlcNAc (blue), and UDP (orange) substrates shown as sticks to illustrate donor/acceptor binding sites.

[Movie S1](#)