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Comparison of Glycosyltransferases GtfA and GtfD for the Biosynthesis of Vancomycin Group Antibiotics: Ligand Binding and Regiospecificity. Anne Mulichak^{1,2}, Wei Lu³, Heather Losey³, Christopher Walsh³, R. Michael Garavito¹; ¹Dept. of Biochemistry & Molecular Biology, Michigan State Univ., East Lansing, MI 48824; ²currently IMCA-CAT, APS, Argonne National Lab, Argonne, IL 60439; ³Dept. of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

Vancomycin group antibiotics are variously glycosylated during biosynthesis by a series of glycosyltransferases (Gtfs), enzymes which offer a potential avenue for the design of new semi-synthetic antibiotics. These structurally homologous Gtfs evolved not only to accept different substrates, but to act with different regiospecificity as well. Notably, GtfD and GtfA from the vancomycin and chloroeremomycin pathways bind the identical acceptor substrate, but transfer L-(epi)vancosamine sugars to different positions on the aglycone. We have determined the crystal structures of GtfD and GtfA as ternary complexes with TDP and the natural acceptor substrate. This work identifies the substrate binding sites, revealing that regioselection is accomplished by dramatically different binding modes for the aglycone in the two enzymes. Conserved Asp13 is identified as the catalytic base, and several additional residues are predicted to be important in donor sugar binding and recognition.