Neutron structure and mutagenic analysis of human ABO bloodgroup glycosyltransferases support an orthogonal associative mechanism of stereospecificity.

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Retaining glycosyltransferase enzymes catalyze the transfer of carbohydrates to either retain or invert product stereochemistry. While the inverting mechanism is mechanistically straightforward — requiring only canonical S_N2 back attack from the acceptor molecule — the mechanism of stereochemistry retention has been a matter of debate. Joint neutron time-of-flight and X-ray crystallographic analysis of human ABO(H) blood group A glycosyltransferase (GTA) present a remarkably aprotic active site that would best facilitate an associative mechanism unlike either contending postulate mechanisms. Such an orthogonal associative (OA) mechanism has significant experimental support: enzymatic activity persists despite alanine mutagenesis of active site residues necessary for previous contending mechanisms, and high-resolution structures exhibit abutting substrates too proximal to permit monosaccharide dissociation. Of all postulated mechanisms, only OA is consistent with all of the available evidence including kinetic isotope effect experiments. OA represents the shortest physical route to glycosyltransfer, avoiding generation of intermediates. It is the simplest of the alternatives and most comparable to uncontested S_N2 mechanisms utilized by inverting enzymes.