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## **Expanding Pyrimidine Diphosphosugar Libraries Via Structure-Based Nucleotidyltransferase Engineering**

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Metabolite glycosylation is effected by three classes of enzymes: nucleotidyltransferases, which activate sugars as nucleotide diphospho-derivatives; intermediate sugar-modifying enzymes; and glycosyltransferases, which transfer the final derivatized activated sugars to aglycon substrates. Last year we determined the first crystal structures of an enzyme responsible for the first step in this cascade, D-glucopyranosyl phosphate thymidyltransferase, or  $E_p$ , from *Salmonella* in complex with product (UDP-Glc) and substrate (dTTP). These structures, in conjunction with the kinetic characterization of  $E_p$ , clarified the catalytic mechanism of this important enzyme class. We have now utilized structure-based engineering of  $E_p$  to produce modified enzymes capable of utilizing "unnatural" sugar phosphates not accepted by wild-type  $E_p$ . In particular, we have designed several mutants which greatly increase the diversity of alpha-D-hexopyranosyl phosphates accepted by  $E_p$ . The crystal structures of three of the mutants were determined to help refine future engineering efforts.