# Supporting Information

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## SI Methods

**Enzyme Activity Assay and Differential Scanning Fluorimetry.** The transferase activity of the wild-type and mutant yeast glycogen synthase 2 (Gsy2p) E169Q enzymes was measured as previously described (1). Protein–ligand solutions were dispensed into 96-well RT-PCR microplates (Roche). The reaction mix included 2.0 μM Gsy2p, 20 mM Hepes (pH 7.5), 0.2 M NaCl, 5× sypro orange, and varying concentrations of either G6P or GCP in a final volume of 25 μL. The GCP titrations were also determined in the presence of 1 mM UMP with and without 0.1 mM G6P. The Roche LightCycler 480 PCR machine with a 96-well thermal block was used and then heated at a rate of 0.06 °C/s for all experiments, $\lambda_{ex} = 483$ nm and $\lambda_{em} = 568$ nm. The melting temperatures were automatically extracted by the Roche protein melting software installed on the LightCycler 480 PCR machine. Further data processing was carried out using the SigmaPlot software package (version 12.3) for nonlinear regression analysis by fitting to the following equation:

$$f1 = \min + \frac{(max - \min)}{\left(1 + \frac{x}{EC50}\right)^{-hillslope}}.$$

**Glucose-1,2-Cyclic Phosphate Synthesis.** Glucose-1,2-cyclic phosphate (GCP) was synthesized by a modification of the method of Zmudzka and Shugar (2) as described by Tagliabracci (3). Briefly, the free acid form of G-1-P was reacted with dicyclohexylcarbodiimide in the presence of pyridine to generate the GCP, which was purified, converted to the barium salt, precipitated from solution, dried to a white powder using a SpeedVac, and stored at −20 °C until use.

1. Thomas JA, Schlender KK, Larner J (1968) A rapid filter paper assay for UDPglucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-14C-glucose. *Anal Biochem* 25(1):486–499.
2. Zmudzka BS, Shugar D (1964) Preparation and chemical and enzymic properties of cyclic phosphates of D-glucopyranose and synthesis of derivatives of N-(D-glucopyranosyl) pyridine. *Acta Biochim Pol* 11(4):509–525.
3. Tagliabracci VS (2010) Metabolism of the covalent phosphate in glycogen. PhD thesis (Indiana Univ-Purdue Univ Indianapolis, Indianapolis).

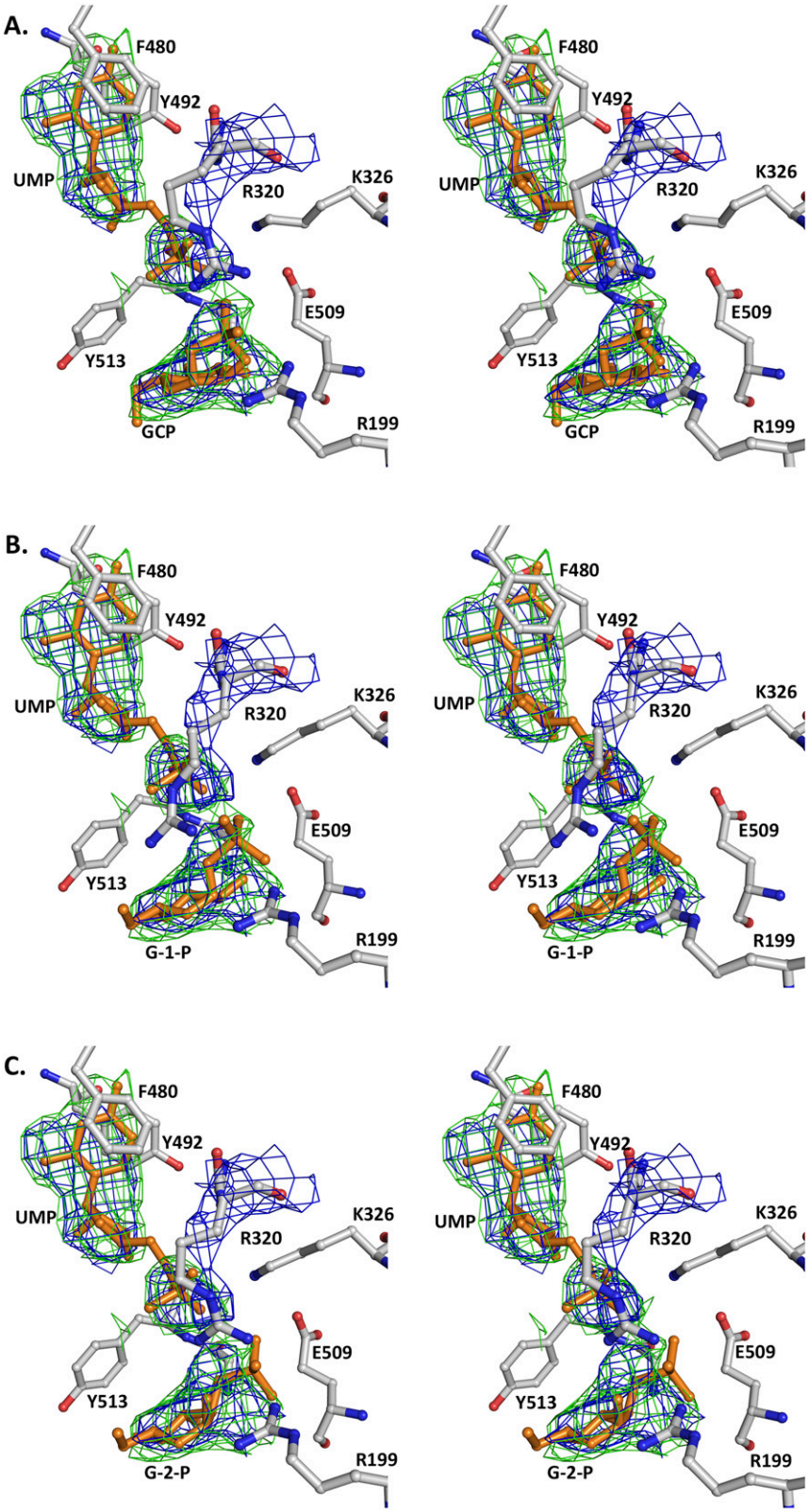


**Fig. S1.** Stereo diagram of the original electron density maps around the bound ligands and R320 in the Gsy2p UDP-glucose binding pocket. The electron density maps were calculated from the models before the addition of ligands, and the σA-weighted $2F_o$-$F_c$ map (contoured at 1.2σ and colored blue) was superimposed with the σA-weighted $F_o$-$F_c$ difference map (contoured at 2.5σ and colored cyan). The refined models of Gsy2p shown as sticks colored in gray are shown in complex with the ligands illustrated in orange. UMP is present in all of the three models and (*A*) GCP, (*B*) glucose-1-phosphate (G-1-P), and (*C*) glucose-2-phosphate (G-2-P).

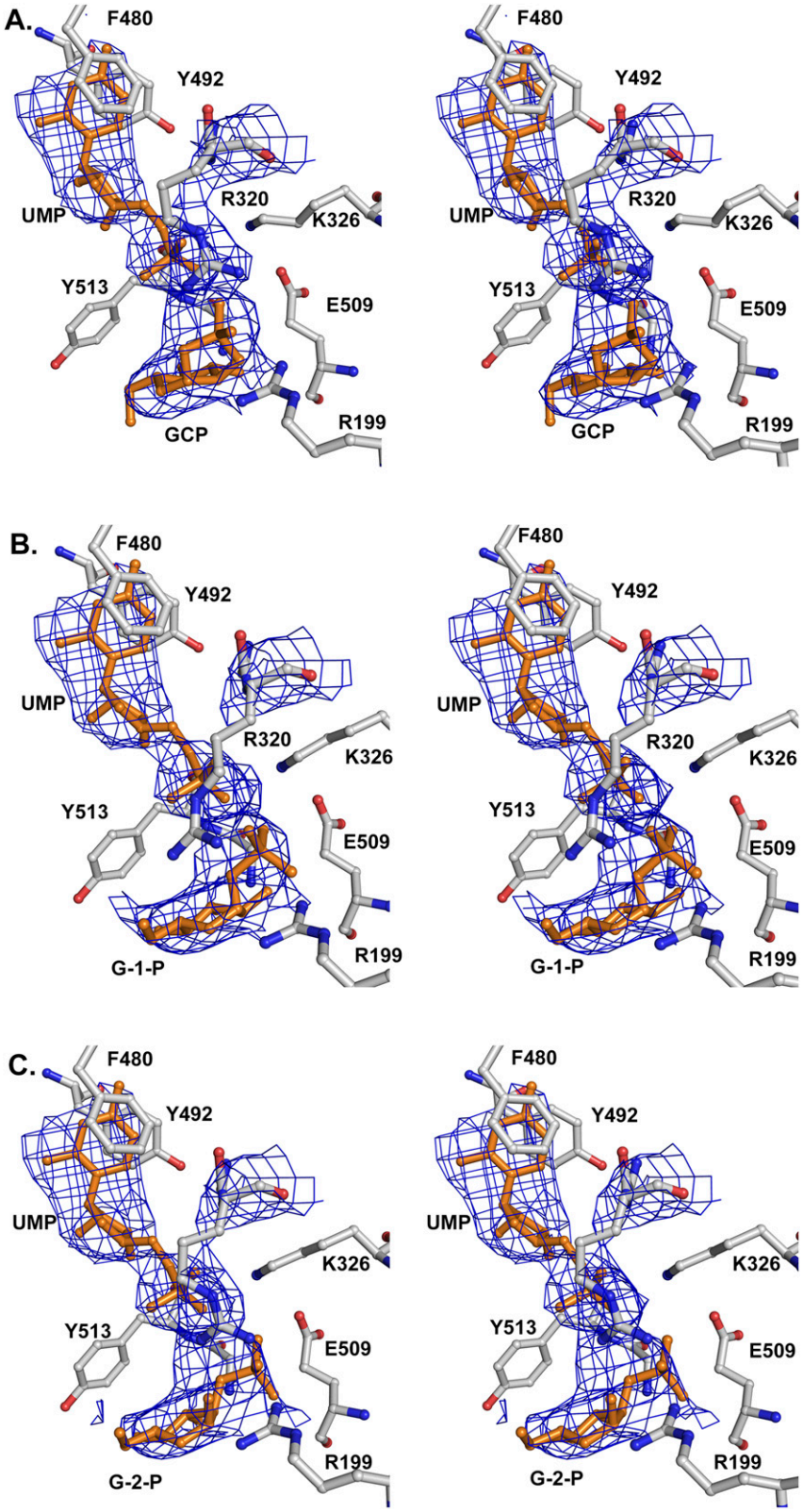


**Fig. S2.** Stereo diagram of the refined electron density maps around the bound ligands and R320 in the Gsy2p UDP-glucose binding pocket. The refined σA-weighted $2F_o$-$F_c$ map (contoured at 1.2σ and colored blue) is shown superimposed with the refined models of Gsy2p with (*A*) GCP, (*B*) G-1-P, and (*C*) G-2-P. The same color scheme used in Fig. S1 was maintained.

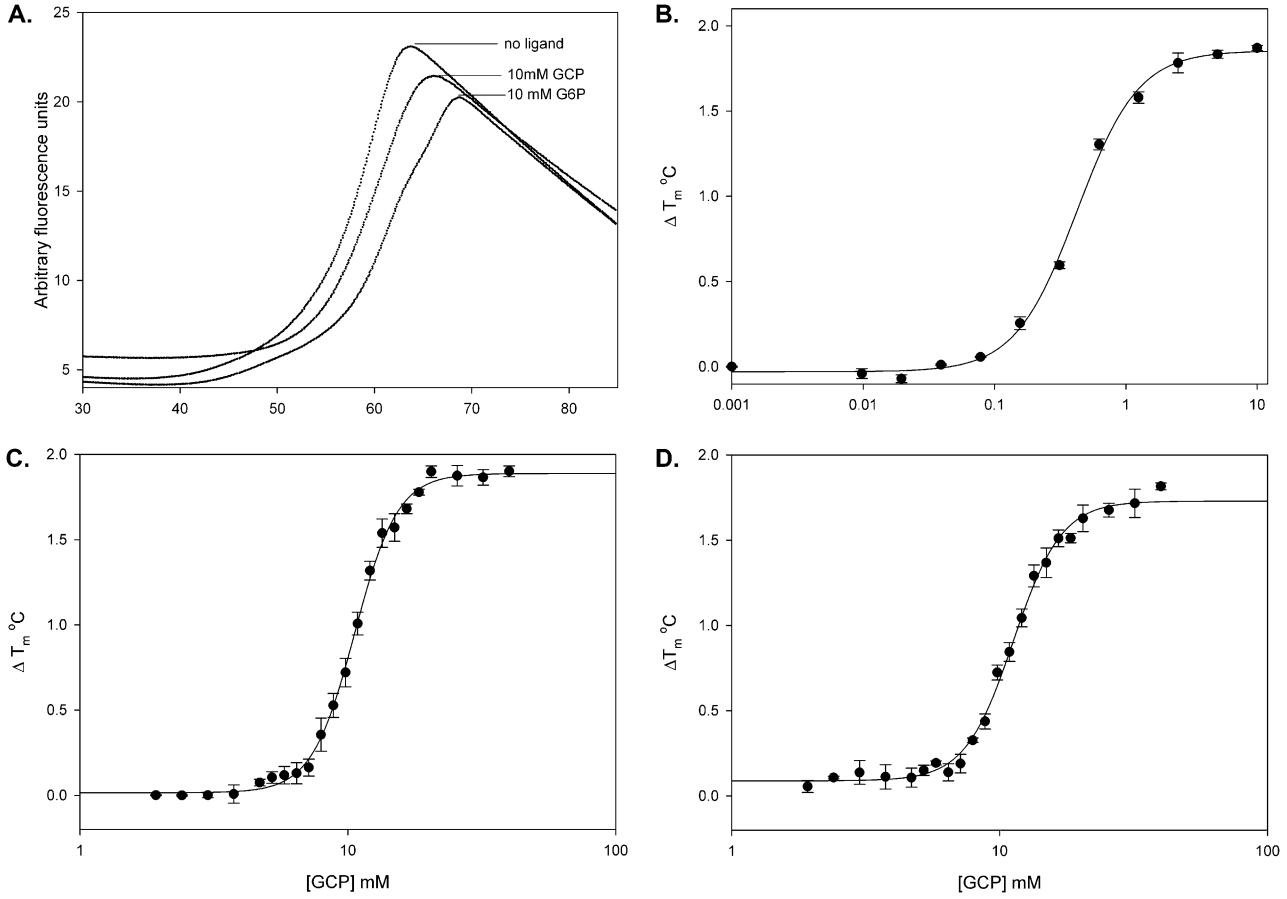


**Fig. S3.** Differential scanning fluorescence results for Gsy2p in the presence of different ligands. (*A*) The raw data are shown for Gsy2p in the absence of any ligands ($T_m$ = 59.2 °C), at 10 mM GCP ($T_m$ = 60.0 °C), and at 10 mM G6P ($T_m$ = 61.2 °C, the allosteric activator of Gsy2p). (*B*) G6P was titrated, and an $EC_{50}$ of 0.42 ± 0.02 mM was determined. (*C*) GCP titration in the presence of 1 mM UMP ($EC_{50}$ = 10.60 ± 0.13 mM) and (*D*) GCP titration in the presence of 1 mM UMP and 0.1 mM G6P ($EC_{50}$ 11.26 ± 0.18 mM).

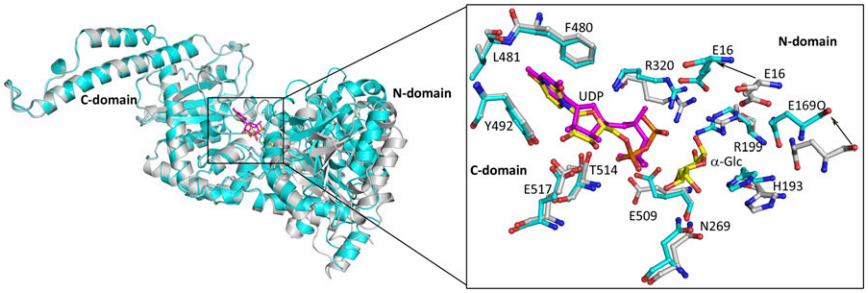


**Fig. S4.** Structural alignment of the Gsy2p E169Q UDP complex (protein in gray, UDP in magenta) with the Gsy2p E169Q UDP·glucose complex (protein in cyan, ligands in yellow). The N-terminal domain of the glucose bound (cyan) structure is 13.3° more closed in the presence of glucose. Superposition of the active sites of the structures is expanded for clarity on the right. The arrows indicate the direction of movement of E16 and E169Q toward the active site upon closure. UDP binds in two conformations within the active site of Gsy2p in which the positions of α- and β-phosphates are interchanged. $UDP_a$ is in yellow (pretransfer conformation), and $UDP_b$ in magenta illustrates the conformation following transfer of glucose.

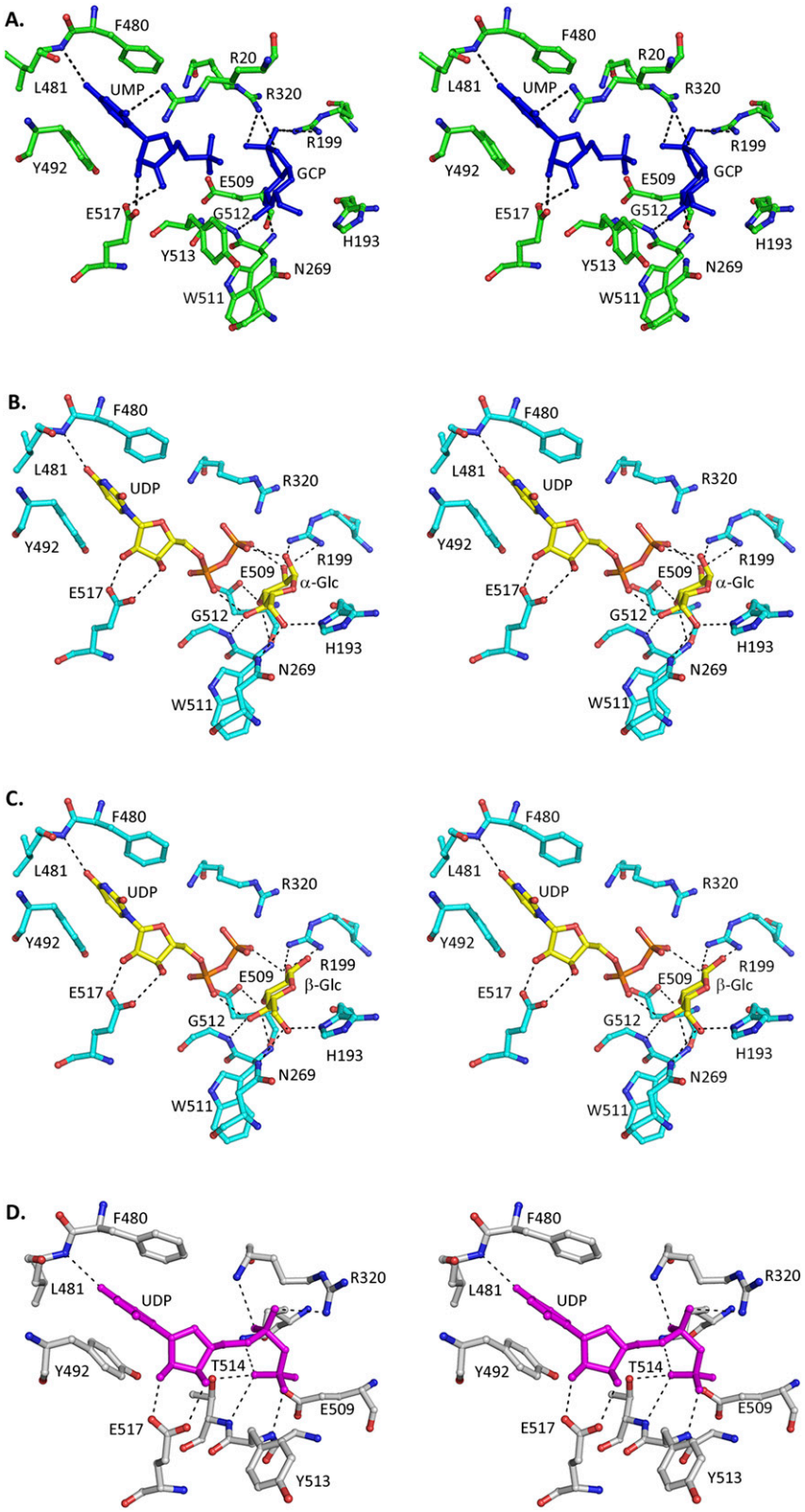


**Fig. S5.** Stereoview representations of the main interactions between the bound substrates and products and surrounding active site residues of Gsy2p. (*A*) The Gsy2pR589/92A·GCP·UMP·G-6-P complex. The active site of the Gsy2pE169Q·Glc·UDP·G-6-P complex with (*B*) β-glucose and (*C*) α-glucose. (*D*) Hydrogen bonding pattern for the posttransfer conformation of UDP active site. The color scheme used in Fig. S3 was maintained. Inferred hydrogen bonds are displayed using black dashes.

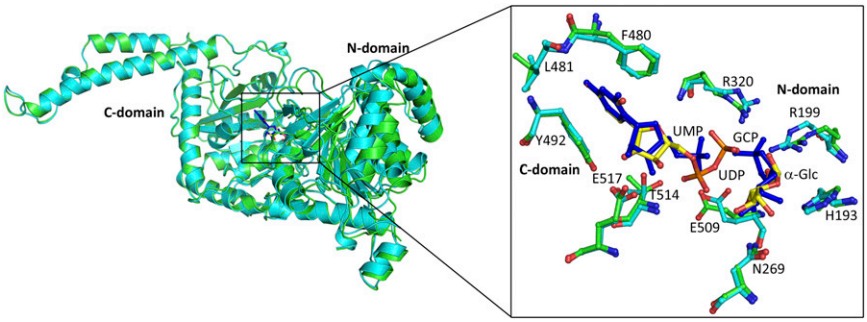


**Fig. S6.** Structural alignment of Gsy2p R589/592A in complex with UMP and GCP. In this representation, the UMP·GCP complex is displayed in green, and ligands are in navy blue, whereas the Gsy2p E169Q complex with UDP·glucose is displayed in cyan, and ligands are in yellow. The N-terminal domain of the glucose bound structure (cyan) is 4.8° more closed in the presence of glucose. The superimposed active sites are expanded for clarity on the right. The C1 atoms of glucosyl moieties are in almost identical positions in the two structures.

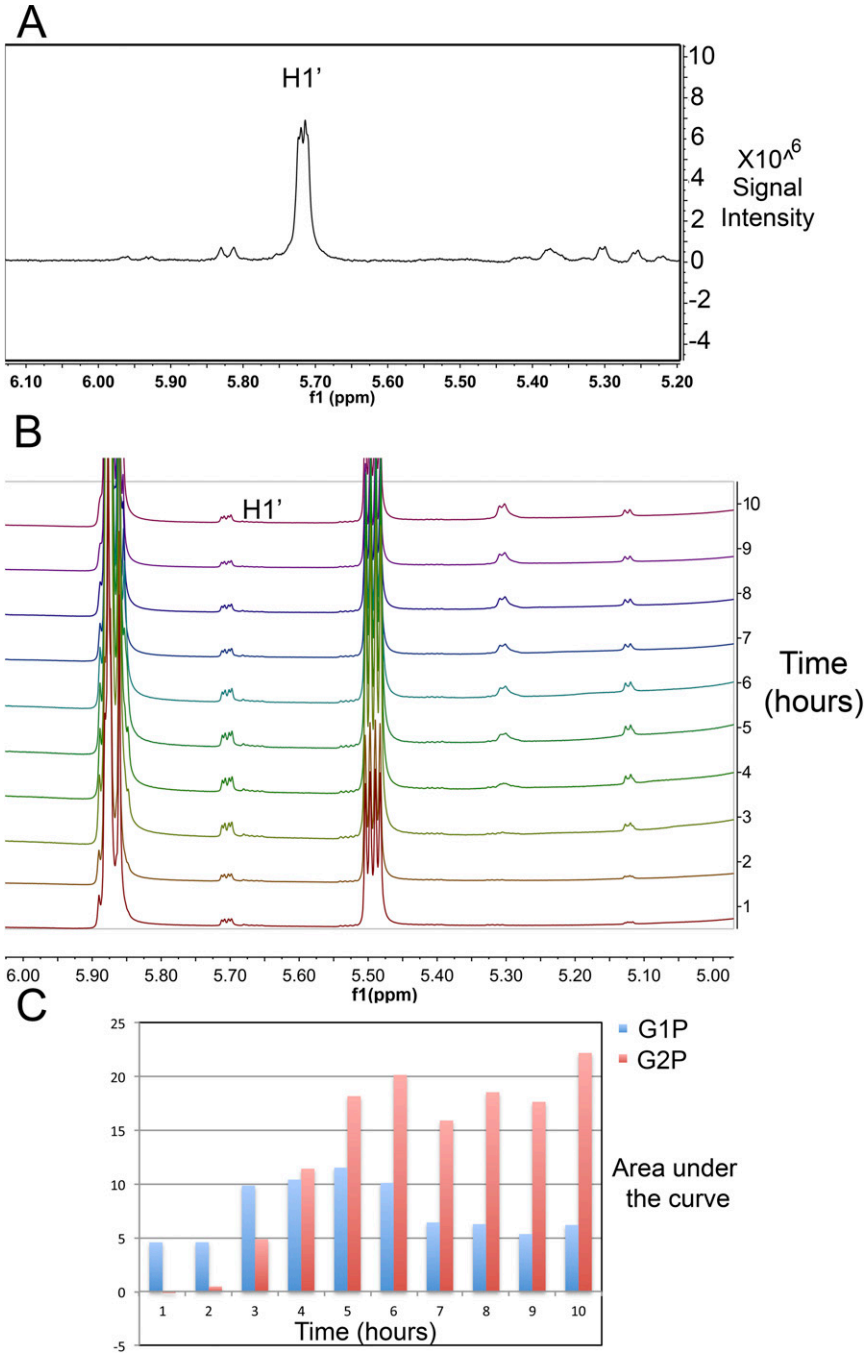


**Fig. S7.** (*A*) $^{1}$H NMR spectrum of synthesized glucose-1,2-cylic phosphate (GCP) with regions from 5.2 to 6.1 ppm highlighted. H1′ resonance appears at 5.72 ppm. (*B*) $^{1}$H NMR spectrum with increasing incubation time of UDP-glucose, Gsy2p, and G6P (concentrations in *Methods*) from 1 h to 10 h. (*C*) Area under the curve for peaks at 5.72 ppm (H1′ GCP, blue) and 5.3 ppm (G2P, red) are plotted as a function of time.

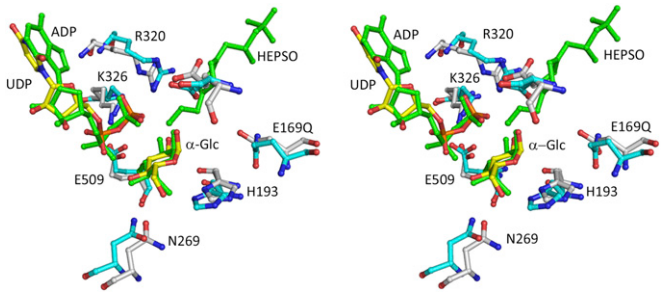


**Fig. S8.** Stereoview representation of the superimposed active sites of the E169Q Gsy2p·UDP·Glc complex (cyan and yellow) with the *Escherichia coli* glycogen synthase·ADP·Glc·HEPSO complex (PDB 2QZS) in gray and green. The residues are labeled according to Gsy2p sequence position. Structural alignment of the active sites was performed in PyMOL.

**Table S1. Summary of regression analysis for differential scanning fluorimetry on Gsy2p in the presence of various ligands**

| | Graph | | |
|---|---|---|---|
| Fit values | B | C | D |
| Ligand | G6P varied | GCP varied + 1 mM UMP | GCP varied + 1 mM UMP + 0.1 mM G6P |
| $R^2$ | 0.996 ± 0.05 | 0.99 ± 0.09 | 0.98 ± 0.09 |
| Max | 1.85 ± 0.02 | 1.89 ± 0.03 | 1.72 ± 0.03 |
| $EC_{50}$ | 0.42 ± 0.02 | 10.60 ± 0.13 | 11.26 ± 0.18 |