

# Supporting Information

Baskaran et al. 10.1073/pnas.1006340107

## SI Text

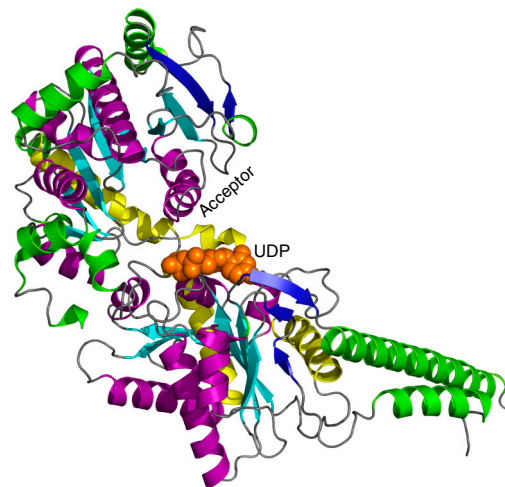
**Intein-Mediated Semisynthetic Enzyme Methods.** The harvested cells (3 L culture) were resuspended in a buffer containing 20 mM Tris-HCl, 500 mM NaCl pH 8.5, 2 mM EDTA, 2 mM benzamidine, 1 mM PMSF and 0.1% Triton X-100. Cells were lysed by French press and the clarified lysate was loaded onto chitin resin (3 mL) preequilibrated with lysis buffer and washed to baseline with the lysis buffer without Triton X-100 (equilibration buffer). For the truncated core enzyme, the column was treated with 10 mL equilibration buffer containing 50 mM DTT. The semisynthetic peptide-ligated proteins were produced by treating the column-bound protein with 2 mL 2% (v/v) thiophenol in equilibration buffer followed immediately by 1 mL of 2 mM synthetic peptide and 2% thiophenol in equilibration buffer. After incubating at 25 °C for 24 hr, Gsy2p was eluted with 20 mL equilibration buffer and 10 mL equilibration buffer containing 50 mM maltose. Gsy2p showed some specific binding with the chitin bead and hence 50 mM maltose was included in the buffer to facilitate more complete elution. Both elution pools were combined and dialyzed against 20 mM Tris pH 8.0 and 1 mM 2-mercaptoethanol. Typical protein yields from 3 L bacterial cultures were 3–5 mg of peptide-ligated Gsy2p. The phosphopeptides and control nonphosphopeptides were synthesized either by the Peptide Synthesis Core facility of Indiana University or Antagene, Inc. The fusion peptides require an N-terminal Cys residue. The wild-type enzyme has a nonconserved Lys at position 642 and this residue was exchanged for Cys in the designed fusion peptides leaving the remainder of the peptide sequences unchanged. The phosphorylated peptide sequences used are as follows (non-phosphorylated controls are identical with the exception of the phosphate attached to Thr668):

642-CKLKVARPLSVPGSPRDLRSNSTVYMT(PO<sub>3</sub>)  
PGDLGTLQ-676

642-CKLKVARPLSVPGSPRDLRSNSTVYMT(PO<sub>3</sub>)  
PGDLGTLQEV NNADDYFSLGVN-690

**Phasing Methods.** The program package SOLVE (1) located a common set of four tantalum cluster sites in each of the two derivatives and a lower occupancy fifth site in one of the datasets. Together these derivatives provided phase information with a mean FOM of 0.47 and a Z-score of 43.8. Density modification in RESOLVE (2) improved these phases using the 4-fold averaging matrix determined from the common four tantalum sites and provided an electron density map to 5.5 Å with a FOM of 0.74. This electron density map showed strong density for many helical tubes and some of the beta-sheets. Phase extension from 5.5 Å to 3.0 Å was accomplished using the program DM as implemented in the CCP4 suite. This phase extension protocol utilized 300 steps from 6.0 Å to 3.0 Å and the same 4-fold averaging matrices utilized by RESOLVE. The resulting 3.0 Å electron density map was marginally interpretable and lacked continuity. However, the general relationship of the protein fold to the bacterial starch synthase enzymes could be identified. To improve the map quality we used the phase combination approach implemented in the program PHENIX (3, 4) (maps\_only subroutine) where a partial model of the yeast Gsy2p monomer was generated by docking elements of secondary structure from a poly alanine model of *Agrobacterium* GS (PDB code 1RZU) into a single subunit within the 3.0 Å electron density map. The tetramer was generated by applying the NCS relations to this partial monomer structure. The phase information from this partial model and the experimental phase information from SOLVE were combined to improve the phase information to 3.0 Å. Two iterations of phase combination with increasingly larger partial models within the program PHENIX produced phases that yielded a completely interpretable 3.0 Å electron density map, which was completely retraced using the program COOT (5). A structure-based sequence alignment of the glycogen synthases assisted in assigning sequence register to the initial poly-alanine model.

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**Fig. S1.** Ribbon diagram of the Gsy2p monomer. The secondary structural elements of the core Rossmann domains are represented in purple and cyan, the linker sequences connecting the two domains are colored yellow and the unique eukaryotic insertions in green and blue. The UDP molecule is represented in orange space filling models and the regions that contributed to glycogen acceptor binding are labeled. [Produced using Pymol (6) for Windows.]



GYS2\_YEAST : --MSR-----D LQNHLLFETA TEV--ANRVCGGIYSVLKSKAPITVAQ--YKDHVHLCPLMKA TYQNEVD LIDWKPRAFSD EHRPUGHALQTHESRCVHFVYCRMLI EGAPRVLFLDLD SV : 111  
GYS2\_DANRE : NLRSPSLSITSLGCLPLFEESLIVPEL L LF EAVAEV--TNKVCGITVVIQTKAKITVDE--WGENVFMHCPYVHEHFKTQVEKCEP FMQ-----AI RAHMDSLINNCQDHF CRMLI BCSFYVLLDFDICA : 123  
GYS2\_HUMAN : NLRGSLSVTSLGCLPQWVEELPVEELL LF EAVAEV--TNKVCGITVVIQTKAKITADE--WGENVFLICPVFHEHMKTQVEKCEP FMD-----AVRAVDAMNKHGCGQHF CRMLI BCSFYVLLDFDICY : 123  
GYS1\_HUMAN : MPNRTLSHSLPGLRDWED E- PD LENSVL F EAVAEV--ANRVCGITVVIQTKAKITGDE--WGDNYFLVGPYTTBQGVRTQVELEA PTP-----ALKRTLD SHNSRCCKVYF CRMLI BCGP LWWLDDVCGAS : 122  
GYS1\_RABIT : MPLSRTLSVSLPGLRDWED E- PD LENSVL F EAVAEV--ANRVCGITVVIQTKAKITGDE--WGDNYFLVGPYTTBQGVRTQVELEA PTP-----ALKRTLD SHNSRCCKVYF CRMLI BCGP LWWLDDVCGAS : 122  
GYS1\_YEAST : --MAR-----D LQNHLLFETA TEV--ANRVCGGIYSVLKSKAPITVAQ--YKDHVHLCPLMKA TYE SEVEKLDWED E SIFPEELLP I QRTLSM SHNSRCCKVYF CRMLI EGAPRVLFLDLD SV : 111  
GYS\_ATU : -----MNVLSVSEIYPLIKTGLADVVGALP LAL EAHGV R--TRT L L P C Y PAVKAAV--TDPVRCF EFTDL LGE-----KAD LLEVQHE-----RLDL L L D L D A P A Y : 88

GYS2\_YEAST : --RCYSEWPKD LWSLVCIPS FEND FETND A I L L C Y T V A W F L G E V A--H L D S Q H A I V A H F H E W L A C V A L P L C R K R--R I D V V I T F T H A T L L G R Y L C A G S C S F D F Y N C L E S V D V D H E A C R F G I Y H R Y C I E R A A H S A D V : 243  
GYS2\_DANRE : --AMND R M R G C D L W S A C C I G L P Y H D R E A M D S L L C S L V A W F F K E L T D Q L Q D R I N V V A H F H E W Q A G T G L V L S R S R--N L P L A T I F T H A T L L G R Y L C A G--M A D F Y M L D K F D I D R E A C E R Q I Y H R Y C L E R A A V H C A H V : 255  
GYS2\_HUMAN : --AMND R M R G C D L W S A C C I G L P Y H D R E A M D S L L C S L V A W F F K E L T D Q L Q D R I N V V A H F H E W Q A G T G L V L S R S R--N L P L A T I F T H A T L L G R Y L C A G--M I D F Y M L D K F N I D K R A C E R Q I Y H R Y C L E R A A H C A H V : 254  
GYS1\_HUMAN : --ANALE R M R G C E L M D T C M I G V W Y D R E A M D A V L F C F L T T W F L C F L A Q G E E R K H V V A H F H E W L A C V L C L C R A R--R L P V A T I F T H A T L L G R Y L C A G--A V D F Y M L N L F N V D V D K R A C E R Q I Y H R Y C L E R A A A H C A H V : 254  
GYS1\_RABIT : --ANALE R M R G C E L M D T C M I G V W Y D R E A M D A V L F C F L T T W F L C F L A Q G E E R K H V V A H F H E W L A C V L C L C R A R--R L P V A T I F T H A T L L G R Y L C A G--A V D F Y M L N L F N V D V D K R A C E R Q I Y H R Y C L E R A A A H C A H V : 254  
GYS1\_YEAST : --RHFLNWEKAD LWSLVCIPS PEH D H E T N D A I L L C Y V V W F L G E V S--K L D S S H A I I G H F H E W L A C V A L P L C R K R--R I D V V I T F T H A T L L G R Y L C A G S F D F Y M L Q Y F D V D Q R A C K R G I Y H R Y C I E R A A A H T A D V : 243  
GYS\_ATU : --YER--SGCP--Y L G Q T G C D Y P D M W K R F A A L S L A A A R I G A--G V L P C W R P D M V H A H W Q A A M T P V Y M R Y A R T P E I P S L L T H E I L A P Q C Q F G A M I F S K--L A L P A H F C M E G I R Y N D V S F L R G G L Q T A T A : 209

GYS2\_YEAST : F T T V S Q I T A F E A E H L-----L R K R P D C I L P N G L N V K F Q A F-----H E F Q N L H A L K R E K I N D F V R G H F C F D F D L D N T L Y F F I A G R Y E F S M R G A D M F I E A L A R L N Y L R V N G S R K T V W A F I W H : 357  
GYS2\_DANRE : F T T V S Q I T A V E A D H M-----L H R N P D V V T P N G L N V K F S A M-----H E F Q N L H S M R S K I Q E F V R G H F Y C H L D F N L E K T L F F F I A G R Y E F S M R G A D L F L E S L S R L N Y L R V N H S D V T V W V F I M : 369  
GYS2\_HUMAN : F T T V S E I T A I E A E H M-----L R K R P D V V T P N G L N V K F S A V-----H E F Q N L H A M Y K A R I Q D F V R G H F Y C H L D F L E K T L F L F I A G R Y E F S M R G A D F L E S L S R L N Y L R N H S D I T V W V F I M : 368  
GYS1\_HUMAN : F T T V S Q I T A I E A Q H L-----L R K R P D I V T P N G L N V K F S A M-----H E F Q N L H A Q S K A R I Q E F V R G H F Y C H L D F N L D K T L Y F F I A G R Y E F S M R G A D V F L E A L A R L N Y L R V N G S R Q T V W A F I M : 368  
GYS1\_RABIT : F T T V S Q I T A I E A Q H L-----L R K R P D I V T P N G L N V K F S A M-----H E F Q N L H A Q S K A R I Q E F V R G H F Y C H L D F N L D K T L Y F F I A G R Y E F S M R G A D V F L E A L A R L N Y L R V N G S R Q T V W A F I M : 368  
GYS1\_YEAST : F T T V S Q I T A I E A E H L-----L R K R P D C I L P N G L N V K F Q A V-----H E F Q N L H A L K R E K I N D F V R G H F C F D F D L D N T V Y F F I A G R Y E F S M R G A D M F I E S L A R L N Y L R V N G S R K T V W A F I M : 357  
GYS\_ATU : L S T V S P S Y A E I L T A E F C M C L E C V I G S R A H V L H C I V N G I D A D V W P A T H L I D H N Y S A M I K N R A L M R K A V A H F R I-----D D D C S P L F C V I S--R L T W Q C G I D L M A R A V D E I V S L-----C G R L V V L G A : 328

GYS2\_YEAST : P A K H N S F T V E A L K Q A E V F A L E N T V H E R V T S I G K R I F D H A I R F P H N G L T E L P D L I G E L L K S S D R V M L K R I L A L R A P E G Q L P P V T T H M L D D A N D L I M K I R Q V Q L F N S P S D R K K H I F H P E F I N A M N P I L G L D Y : 492  
GYS2\_DANRE : P A K T N N F N V E L R K Q A V R K Q L M D T A Q S V R K P G R G L Y E S L L R G-----E I P--D M S K I L D R D D P T I M K R A I Y A T Q R H--S L P P V T T H M L D D S T D P L S C N I R R I G L F N S R N D R K V I F H P E F L S S T S P L L P M D Y : 494  
GYS2\_HUMAN : P A K T N N F N V E L R K Q A V R K Q L M D V A H S V R K P G R G L Y E A L L R G-----E I P--D L M D I L D R D D P T I M K R A I Y A T Q R Q--S L P P V T T H M L D D S T D P L S T I R R I C L F N S S A D R K V I F H P E F L S S T S P L L P M D Y : 493  
GYS1\_HUMAN : P A K T N N F N V E L R K Q A V R K Q L M D T A M T V R K R P G R G L Y E S L L V C-----S L P--D M N R L D K E D F T H M K R A I F A T Q R Q--S F P P V C T H M L D D S S D P L I T T I R R I C L F N S S A D R K V I F H P E F L S S T S P L L P M D Y : 493  
GYS1\_RABIT : P A K T N N F N V E L R K Q A V R K Q L M D T A M T V R K R P G R G L Y E S L L V C-----S L P--D M N R L D K E D F T H M K R A I F A T Q R Q--S F P P V C T H M L D D S S D P L I T T I R R I C L F N S S A D R K V I F H P E F L S S T S P L L P M D Y : 493  
GYS1\_YEAST : P A K T N S T V E A L K S A I V K S L E N T V M E V T A S I G K R I F E H T M R Y P H N G L E S E L P T N I D E L L K S S E K V L L K R V L A L R P Y C E L P P V W T H M L C D D A N D P I L M Q I R V R L F N D S D R K V I F H P E F I N A M N P I L G L D Y : 492  
GYS\_ATU : C D-----V A L E G A L L A A S R H H C-----R V G V A I G Y N E-----P L S : 359

GYS2\_YEAST : D E F V R G C H L G V P P S Y Y E P M C Y T P A C T V M G P S I T T N S C F G A Y M E D L E I T M Q A K-----D Y G I Y I V D R R F S A P D E S V E Q L W D Y M E E F V K K T B R Q R I N Q M R T E R L S D L L D W K R M C L E Y V K A P Q L A L R E C Y P : 619  
GYS2\_DANRE : E F V R G C H L G V P P S Y Y E P M C Y T P G C R T W M G I P S V T I N L S C F C C F M E H H V S D P S E-----Y G I Y I V D R R F S A D E S C M L T Q M F S F C Q K S R R Q R I Q M R T E R L S D L L D W R Y L G R F Y M A R H L A L S S F S P : 619  
GYS2\_HUMAN : E F V R G C H L G V P P S Y Y E P M C Y T P A C T V M G I P S V T I N L S C F C C F M E H H V A D P T A-----Y G I Y I V D R R F S A D E S C M L T K F L Y G F C Q K S R R Q R I Q M R T E R L S D L L D W R Y L G R Y Q H A R H L T L S R A F P : 618  
GYS1\_HUMAN : E F V R G C H L G V P P S Y Y E P M C Y T P A C T V M G I P S I T N L S C F C C F M E H H I A D P S A-----Y G I Y I L D R R F S A D E S C S Q L T S F L Y S F C Q K S R R Q R I Q M R T E R L S D L L D W K Y L G R Y Y M S A R H M A L S K A F P : 618  
GYS1\_RABIT : E F V R G C H L G V P P S Y Y E P M C Y T P A C T V M G I P S I T N L S C F C C F M E H H I A D P S A-----Y G I Y I L D R R F S A D E S C S Q L T S F L Y S F C Q K S R R Q R I Q M R T E R L S D L L D W K Y L G R Y Y M S A R H M A L A K A F P : 618  
GYS1\_YEAST : D E F V R G C H L G V P P S Y Y E P M C Y T P A C T V M G P S I T T N S C F G A Y M E D L E I T D Q A K-----D Y G I Y I V D R R F S A P D E S V E Q L W D Y M E E F W N K T B R Q R I N Q M R T E R L S D L L D W K R M C L E Y V K A P Q L C L R A P P E : 619  
GYS\_ATU : H L M Q A C D A I I T P S R F E P C G I L Q L Y A L R Y C C I P V A R T G C L A D T V T-----D A M H A A L A S K A A T--G V Q F S P-----V T I D C L R Q A I R T R V Y T H D--P K L W T Q M Q K L G M K S D V S--W E R S A G L Y A--A L Y S Q L T S K I G H : 480

GYS2\_YEAST : D Q F R E L V C E E L N D S N D A L A G C K R--L R V A R L S V P C S P R D L R E N S T V Y I T P C D L C T L Q E V N-----N A D D Y-----F S L G V N-----P A A D D D D-----G P Y A D D S : 705  
GYS2\_DANRE : E K F R P E H-----L N L T S T Q C-----F R Y P R P S S V P P S P S A S I H-----S T P H S D E E D D T Y D E E E E A E D R L N I K A P-----F S V G A D T D G K R T Q P V E N G--N----- : 701  
GYS2\_HUMAN : D K F H-----V E L T S P P T E G F R Y P R P S S V P P S P S C S Q A-----S S P Q S D V E D E V E-----D E F Y D E E E R A E R D R L N I K S P F S L S H V P H G K K L H C E Y K--N----- : 703  
GYS1\_HUMAN : E H F T-----Y E P N E A D A A Q G Y R Y P R P A S V P P S P S L S R H-----S S P H Q S E D E D P K N G L E E D C E R Y D E E A A A D R M N I R A P E W P R A S C T S S T S G K R--N S V D T A T S S L S T P S E P L S P T S L G E E R N : 737  
GYS1\_RABIT : D H F T-----Y E P H E A D A T Q G Y R Y P R P A S V P P S P S L S R H-----S S P H Q S E D E E E P K N G L P E D C E R Y D E E E A A A D R M N I R A P E W P R A S C T S S S G C S K R N S V D T--N-----S S L S T P S E P L S P A S L G E E R N : 735  
GYS1\_YEAST : R Q P K V Q L G E T I S D A M M T L A G C K R--F K I A R P L S V P C S P K--V R S N S T V Y I T P C D L C T L Q D A N-----N A D D Y-----F A L S T N-----G A I D N D D D D N D T S A Y Y E D--N : 708  
GYS\_ATU : ----- : -

Fig. 53. Glycogen synthase sequence alignment. Structure-based sequence alignment of the Agrobacterium GS enzyme with the representative GS sequences of the eukaryotes. Residues in red, blue, and green represent 100%, 80%, and 60% sequence conservation respectively. The multiple sequence alignment of GS was generated using clustalW (8). The structure-based alignment of Agrobacterium and yeast monomers was generated using LSQ superpose in Coot (5). This alignment was used as the basis to manually edit the multiple sequence alignment produced by the program GeneDoc (9).

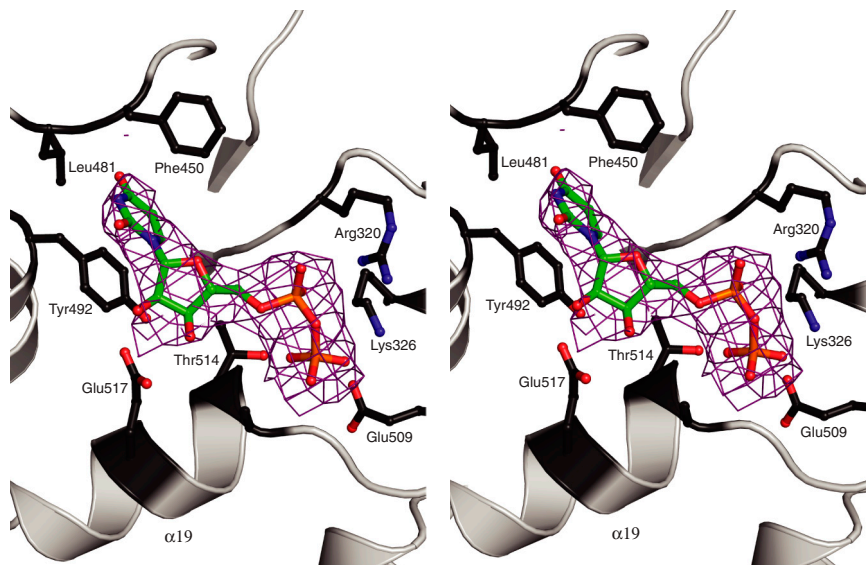


Fig. 54. Stereo diagram of the Gsy2p UDP binding pocket. Ribbon representation of the Gsy2p UDP binding pocket with the bound UDP and interacting residues in ball and stick model. The map shown is the original 2Fo-Fc electron density map for the bound UDP prior to its inclusion in the model (contoured at 1 standard deviation of the map). The residues involved contacting the UDP molecule are labeled. The uridine ring is sandwiched between the aromatic side chains of Phe480 and Tyr492, which is part of the  $\beta$ 15- $\alpha$ 18 loop whose interactions across the subunit interfaces change upon glucose-6-phosphate binding. The helical dipole of helix  $\alpha$ 19 and the side chains of Arg320 and Lys326 are positioned to stabilize the UDP leaving group. The proposed nucleophile (Glu509) (10) is positioned near the  $\beta$ -phosphate of UDP to interact with the donor glucose moiety of UDP-glucose, while the proposed general base (Glu517) (10) is instead positioned to form hydrogen bonds with both the 2' and 3' hydroxyls of the ribose moiety. [Produced using Pymol (6) for Windows.]

