Supporting Information

Structural Basis for Regulation of Human Glucokinase by Glucokinase Regulatory Protein

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Table S1: Data statistics and refinement details

Data collection	
Wavelength (Å)	1.00
Space group	$P2_12_12_1$
Unit cell dimensions [*a, b, c* (Å)]	95.66, 105.31, 132.41
Resolution range (Å)	48.93–3.40
highest resolution shell (Å)[a]	3.50–3.40
No. of observed reflections	123,638
No. of unique reflections[b]	18,950 (1843)
Multiplicity	6.50 (6.23)
Completeness (%)	99.9 (99.7)
<I/σI>	12.93 (1.73)
R_{merge} (%)	7.12 (69.9)
R_{meas} (%)	7.76 (76.3)
Wilson B-factor	136.76
Refinement	
R_{work} (%)	24.4
R_{free} (%)	29.2
No. atoms	7699
macromolecules	7682
Ligands	17
Water	0
B-factor (Å^2)	95.5
macromolecules	95.5
Ligands	101.1
R.m.s. deviations	
bond lengths (Å)	0.006
bond angles (deg)	0.96
Ramachandran statistics (%)	
Favored	95.3
Outliers	0.2
Molprobity score	1.57

[a]Values in parentheses are for the highest resolution shell. As described above, the resolution of 3.50 Å was determined with an <I/σI> criterion of 2, but data up to 3.40 Å were used during refinement.

[b]Value in parentheses indicates number of reflections used for R_{free} calculation.

Figure S1. Purification and crystallization of the GCK-GKRP-fructose 6-phosphate complex. (A) Size exclusion chromatograms of isolated human pancreatic GCK (blue), isolated rat liver GKRP (green) and the stable GCK-GKRP-fructose 6-phosphate complex (red). (B) SDS-PAGE analysis of the higher molecular weight peak (*) demonstrates the presence of both proteins in the complex. (C) Representative crystal of the complex.

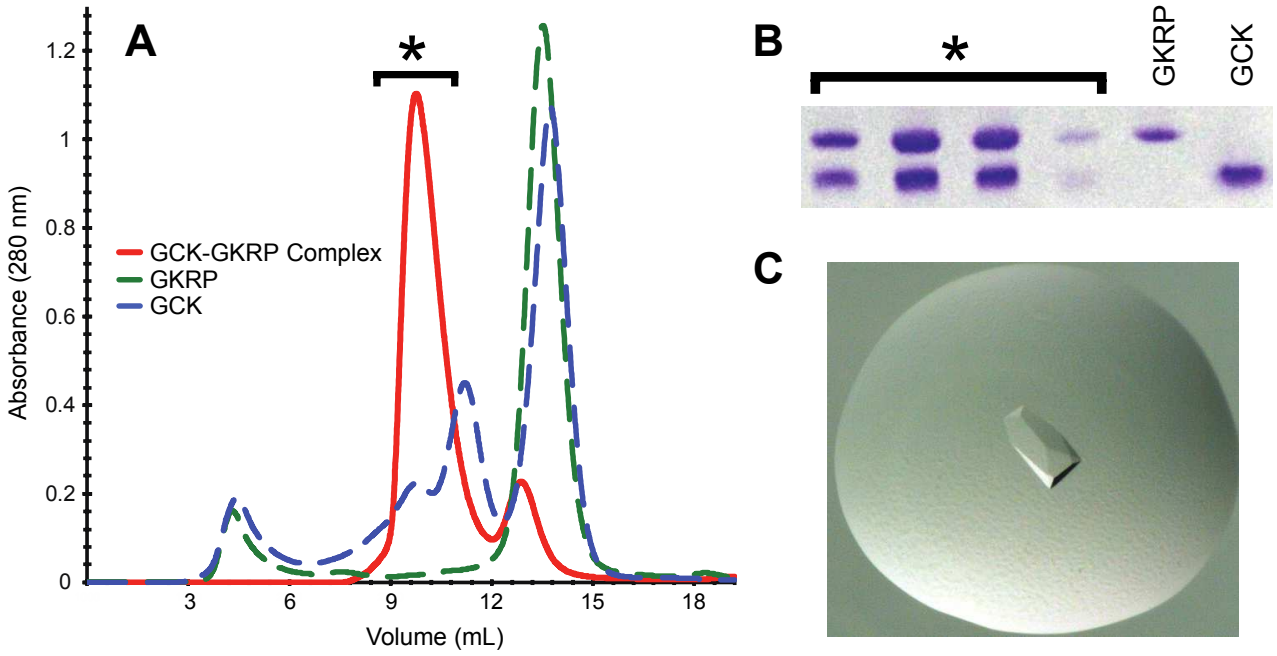

Figure S2: Electron density map after molecular replacement with a partial search model. To ensure that the structure was properly solved and to confirm correct placement of the small domain of GCK, an additional molecular replacement trial was carried out with the "capless" GKRP model and only the large domain of GCK, omitting the small domain of GCK from the search model. The electron density map below is shown at 1.2 σ (blue) and the difference electron density map is shown at 3.5 σ (green/red). The final refined structure is shown as tubes: light blue depicts the large domain, dark blue the small domain of GCK. Since the small domain of GCK was omitted during this particular molecular replacement trial, the electron density (blue and green) after molecular replacement clearly indicates that the small domain of GCK adopts the open conformation, as present in the refined model.

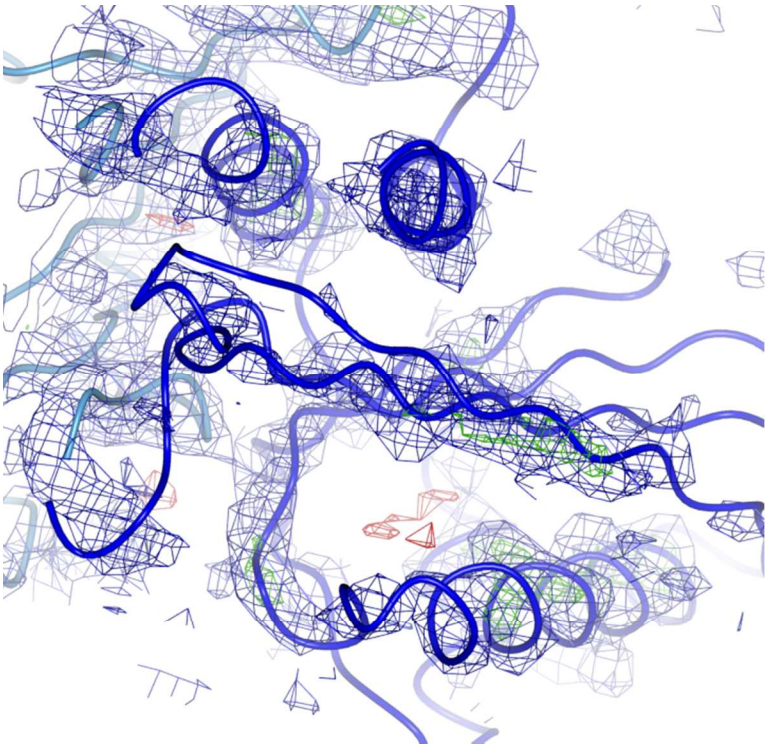

Figure S3. Electrostatic potential depiction of the GCK-GKRP interaction interface. (A) Electrostatic potential of the complex with GCK and GKRP (same orientation as Figure 1). (B) Electrostatic potential of GCK after rotation of 120 degrees to show the interface. (C) Electrostatic potential of GKRP after rotation of 120 degrees to show the interface. All electrostatic potentials are depicted from -5 to 5 kT/e projected on the solvent-accessible molecular surface. Note the electrostatic complementarities surrounding the interaction interface.

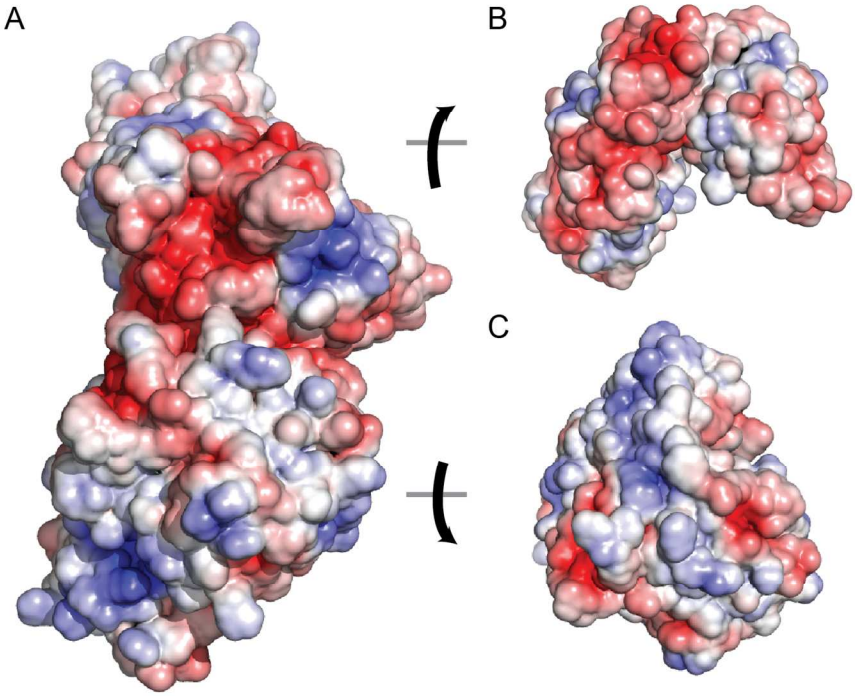

Figure S4. Distribution of the thermal displacement parameters in the GCK-GKRP-F6P complex (same orientation as Figure 1). Fructose 6-phosphate is shown in grey (space-filling). Regions with low thermal displacement parameters (around 50 $Å^2$) are depicted as tubes with small diameter (blue), regions with high thermal displacement parameters (around 160 $Å^2$) as tubes with large diameter (red). The cap domain does not show a rigid conformation; however, the interface region between GCK and GKRP shows low thermal displacement factors, indicative of a fixed conformation.

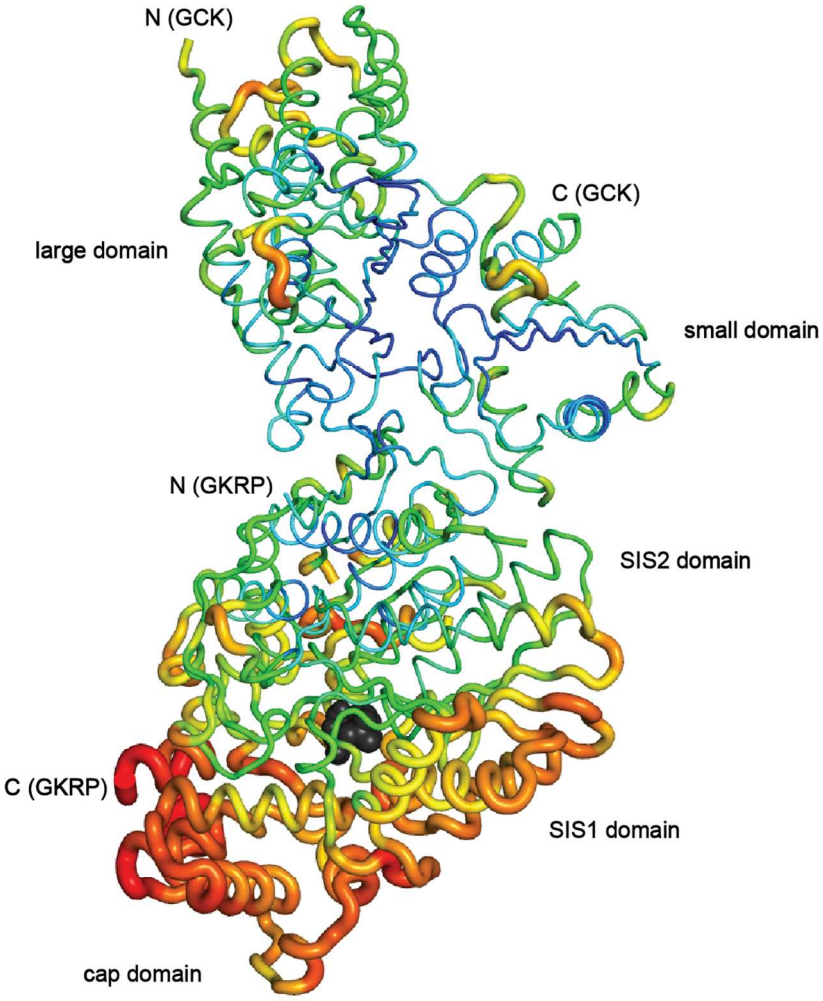

Figure S5. Overlay of the fructose phosphate binding sites in isolated GKRP (fructose 1-phosphate, grey, PDB 4BB9) and GKRP from the GCK-GKRP complex structure (fructose 6-phosphate, green). Residues that interact with fructose 1-phosphate in the isolated structure are depicted as sticks (SIS1 domain in orange, SIS2 domain in red, cap domain in pink, isolated GKRP in grey). For this figure, alignment of the two structures is based on the interacting residues. The residues from SIS1 domain (orange) superimpose with the respective residues from the isolated structure (phosphate binding site). The residues from the SIS2 and cap domains do not overlap as closely, consistent with the structural rearrangement of both domains.

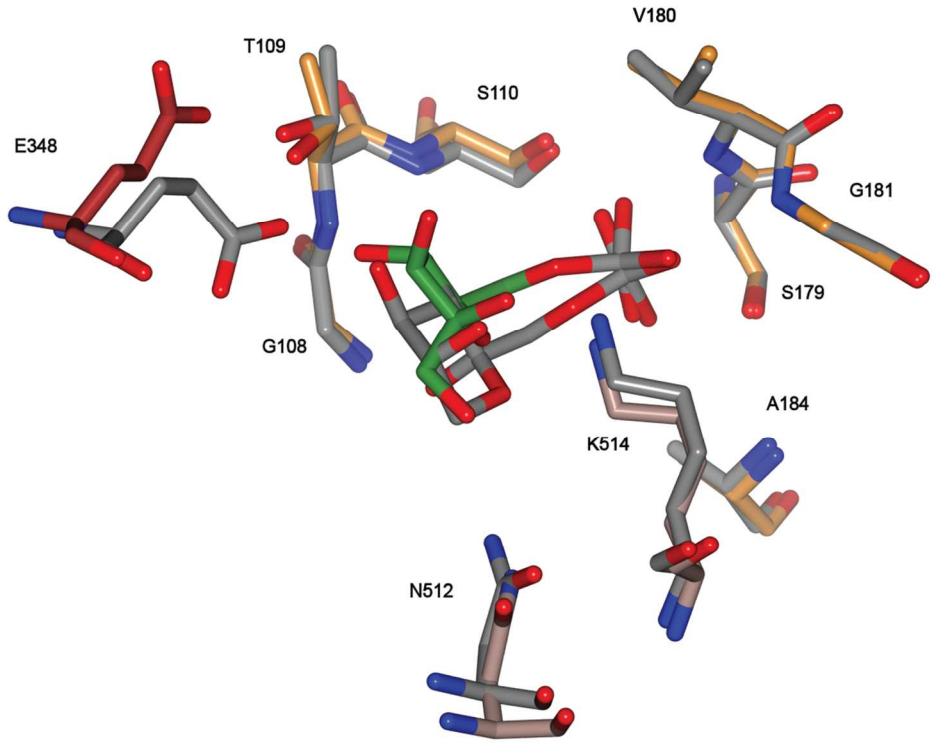

Figure S6. The N-terminus of GKRP reorients in the complex structure. Due to the movement of the cap domain, the N-terminus does not interact with the cap of GKRP as in the isolated structure (iGKRP, grey, PDB 4BB9). Instead, the N-terminus of GKRP in the complex (cGKRP, yellow) makes contacts with GCK residues 103-109 and 452-460 of the symmetry-related molecule (green).