

Supplemental Information

Figure 1: Effect of glucose and ATP on the thermal unfolding of 5.54 μM GK in 50 mM HEPES buffer pH 8.0, 25 mM KCl, 2.0 mM TCEP, 1.0% DMSO. GK alone, solid black line; GK with 100 mM glucose, dotted blue line; and GK with 10 mM ATP/MgCl₂, dashed red line. The vertical lines represent the respective thermal midpoint of the transitions as obtained from fitting to a non-2-state zero heat capacity change model.

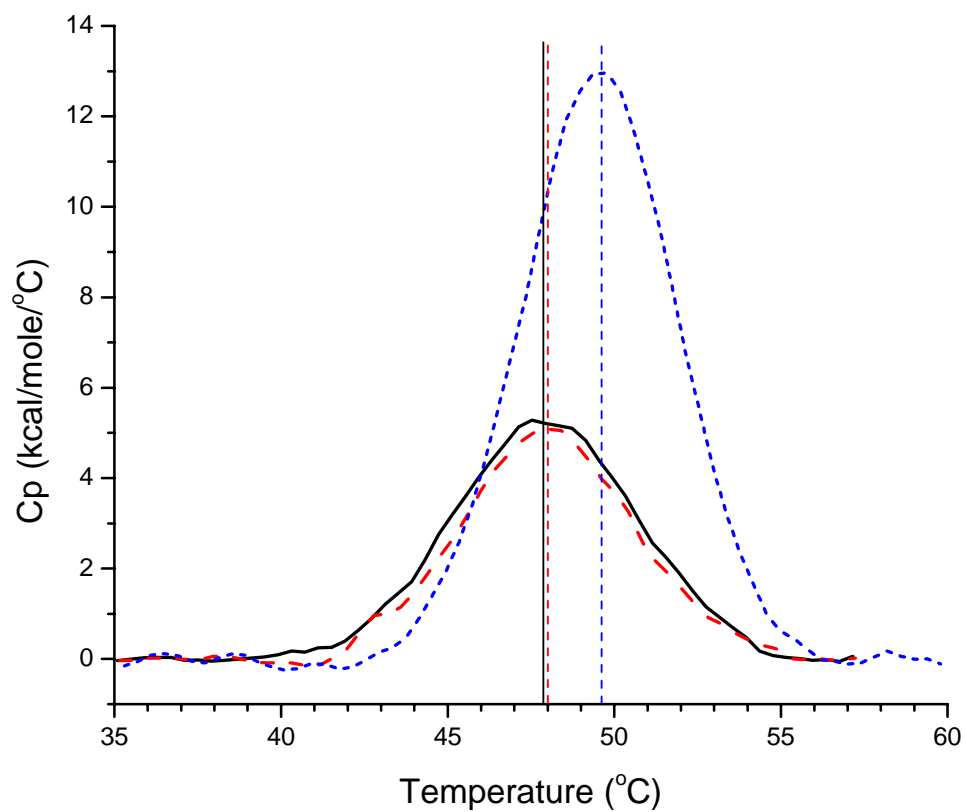
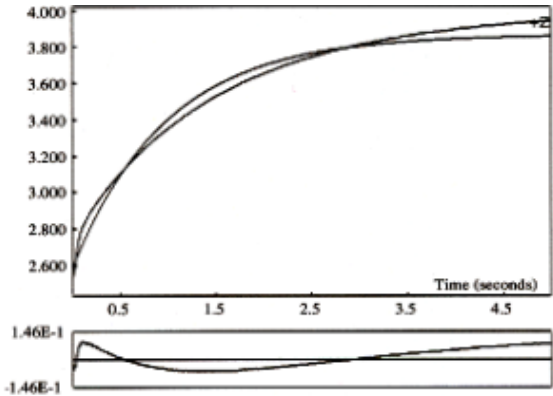
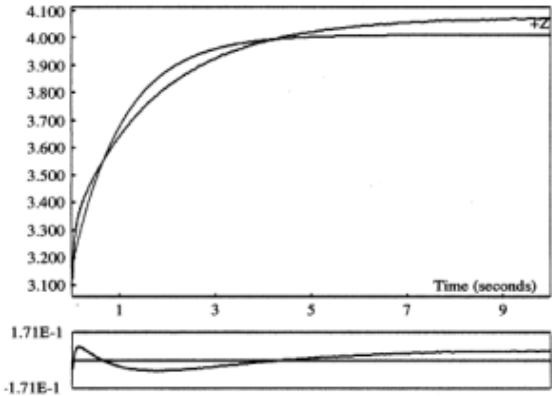


Figure 2: Single exponential fitting to (A) glucose binding to GK WT, (B) 2-deoxyglucose binding to GK WT and (C) glucose binding to Y214C.

A.



B.



C.

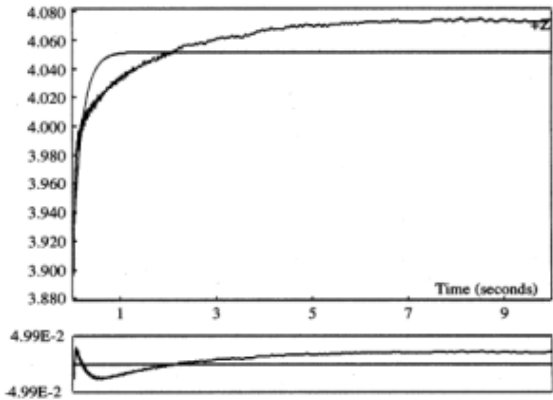
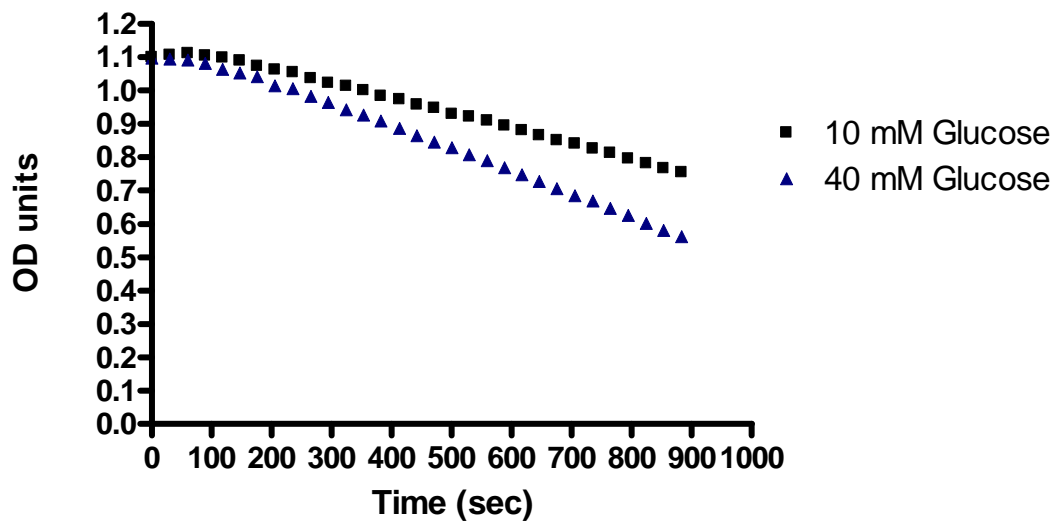


Figure 3: Time course using the PK/LDH coupled assay system (A) No GK incubation with glucose, assay was initiated by addition of 10 nM GK (B) GK incubation with glucose, assay was initiated by addition of ATP.

A.



B.

