Glucose modulation of glucokinase activation by small molecules - SUPPORTING
INFORMATION.

Erik C. Ralph, Jim Thomson, Jonathan Almaden, and Shaoxian Sun

SI Figure 1. Representative DSC traces of GK in the absence of glucose and activators (a), in the presence of 100 μ M GKA1 (b), 100 mM glucose (c) and both 100 μ M GKA1 and 100 mM glucose (d). All scans were done in 50 mM HEPES, 25 mM KCl, 2 mM TCEP, pH 8.0 containing 7 μ M enzyme and 1% DMSO.

SI Figure 2. Titration of GKA1 to GK in the presence of 100 mM glucose monitored by ITC.

Conditions were as described in the Experimental Procedures.

SI Figure 3. Scintillation counts for GKA1 binding as measured by SPA in the absence of glucose (A) and presence of 5 mM glucose (B). Conditions were as described in the Experimental Procedures. The data obtained in the absence of GK ($\diamond$) represented the non-specific binding of ^3H -GKA1 and were fit to a linear relationship. The enzymatic data ($\bullet$) were fit to a sum of equation 3 and a linear slope. The corrected data ($\circ$) obtained by subtracting the non-specific binding from the enzymatic data were fit to equation 3. The corrected data for zero glucose were not fit in (A). The dotted line depicts zero *CPM*.

SI Table 1. GK activation by activators in the absence and presence of 0.2 μM GKRP.

Activator	GKA1		GKA2		GKA3	
GKRP	-	+	-	+	-	+
v_0, s^{-1}	17	2.2	15	1.9	15	1.6
v_m, s^{-1}	30	30	21	20	32	31
$EC_{50}, \mu\text{M}$	0.31	5.0	2	16	0.93	15

Assay conditions were as described in the experimental section. The values were obtained by fitting the observed rates to equation 2.

SI Scheme 1. Representative structures of GK activators previously reported.

Reference 10

Reference 9

Reference 13

Reference 13

Reference 12

Reference 17

Appendices

A: Derivation of Equations 5

When using the SPA method, the monitored signal (*CPM*) is the result of activator binding to the enzyme, and is therefore proportional to the concentration of enzyme present in an activator bound form (Scheme 1). The *CPM* is related to the activator-bound enzyme concentration by factor *C*, which accounts for the molar ratio of tritium labeled GKA1 and its specific activity. This relationship can be expressed as shown in equation S1.

$$\frac{CPM}{C} = \frac{[E_A] + [E_A \cdot Glc]}{[E^*] + [E] + [E^* \cdot Glc] + [E \cdot Glc] + [E_A] + [E_A \cdot Glc]} \quad [\text{Eq S1}]$$

Expressing all terms as a ratio relative to $[E_A]$ yields equation S2.

$$\frac{CPM}{C} = \frac{1 + \frac{[E_A \cdot Glc]}{[E_A]}}{\frac{[E^*]}{[E_A]} + \frac{[E]}{[E_A]} + \frac{[E^* \cdot Glc]}{[E_A]} + \frac{[E \cdot Glc]}{[E_A]} + \frac{[E_A]}{[E_A]} + \frac{[E_A \cdot Glc]}{[E_A]}} \quad [\text{Eq S2}]$$

These terms are related to the equilibrium constants as defined below. Substitution of the equilibrium constants into equation S2 and subsequent algebraic rearrangement yield equation S3.

$$K_1 = [E^*][Glc]/[E^* \cdot Glc]; K_2 = [E^* \cdot Glc]/[E \cdot Glc]; K_3 = [E^*]/[E]; \\ K_4 = [E][Glc]/[E \cdot Glc]; K_5 = [E \cdot Glc][A]/[E_A \cdot Glc]; K_6 = [E][A]/[E_A]; \\ K_7 = [E_A][Glc]/[E_A \cdot Glc].$$

$$\frac{CPM}{C} = \frac{1 + [Glc]/K_7}{1 + \frac{K_3 K_6 + K_6}{[A]} + [Glc] \frac{K_2 K_5 + K_5 + [A]}{[A] K_7}} \quad [\text{Eq S3}]$$

From here, it is very useful to rearrange the equation into the form of equation S4. This equation can be used to describe any hyperbolic curve with a non-zero intercept (C_1) and a plateau (C_2) at

saturating concentrations of X . K refers to the apparent K_D such that when X is equal to the K , the observed Y is equal to the average of C_1 and C_2 .

$$Y = \frac{C_1 + C_2 \frac{[X]}{K}}{1 + \frac{[X]}{K}} \quad [\text{Eq S4}]$$

Using this format for equation S3 where X is glucose, yields equation S5.

$$\frac{CPM}{C} = \frac{\left(\frac{[A]/(K_3K_6 + K_6)}{1 + [A]/(K_3K_6 + K_6)} \right) + \left(\frac{[A]/(K_2K_5 + K_5)}{1 + [A]/(K_2K_5 + K_5)} \right) \left(\frac{[Glc]}{\frac{[A]K_7 + K_3K_6K_7 + K_6K_7}{K_2K_5 + K_5 + [A]}} \right)}{1 + \frac{[Glc]}{\left(\frac{[A]K_7 + K_3K_6K_7 + K_6K_7}{K_2K_5 + K_5 + [A]} \right)}} \quad [\text{Eq S5}]$$

The apparent $K_{D, \text{Glc}}$ value is defined by equation S6.

$$K_{D, \text{Glc}} = \frac{[A]K_7 + K_3K_6K_7 + K_6K_7}{K_2K_5 + K_5 + [A]} \quad [\text{Eq S6}]$$

Finally, using the thermodynamic relationships of $K_6K_7 = K_4K_5$ and $K_3K_4 = K_1K_2$, and subsequent algebraic rearrangement yields equation 5.

$$K_{D, \text{Glc}} = \frac{\frac{K_1K_2 + K_4}{1 + K_2} + (K_7) \frac{[A]}{K_2K_5 + K_5}}{1 + \frac{[A]}{K_2K_5 + K_5}} \quad [\text{Eq 5}]$$

B: Derivation of Equation 6.

Equation S7 is obtained by rearranging equation S3 into the form of S4 where X is the activator. In this case, C_1 is zero since no signal is expected in the absence of activator. C_2 is 1, because all of the enzyme will be in an activator-bound form at infinite GKA concentrations.

$$\frac{CPM}{C} = \frac{[A]}{1 + [A] \left(\frac{[Glc] + K_7}{K_6 K_7 + K_3 K_6 K_7 + [Glc](K_5 + K_2 K_5)} \right)} \quad [\text{Eq S7}]$$

The $K_{D, \text{GKA}}$ value is defined by equation S8.

$$K_{D, \text{GKA}} = \frac{K_6 K_7 + K_3 K_6 K_7 + [Glc](K_5 + K_2 K_5)}{[Glc] + K_7} \quad [\text{Eq S8}]$$

Dividing all terms in equation S8 by K_7 yields equation 6.

$$K_{D, \text{GKA}} = \frac{(K_6 + K_3 K_6) + (K_5 + K_2 K_5) \frac{[Glc]}{K_7}}{1 + \frac{[Glc]}{K_7}} \quad [\text{Eq 6}]$$

C: Kinetic simulations

SI Scheme 2.

SI Scheme 2 shows the model used for kinetic simulation. The equilibrium constants are numbered K_1 - K_{12} , with glucose (G) or activator (A) binding, product (P) formation, product release, and enzyme activation ($E^* \rightarrow E$) defined as forward rate constants (k_{forward}). Reverse reactions are defined by reverse rate constants (k_{reverse}). ATP binding, chemistry, and the first product release are combined into one irreversible step, represented by K_8 or K_9 . Simulations were done using KINSIM [Barshop, B. A., Wrenn, R. F., and Frieden, C. (1983) *Anal. Biochem.* 130, 134-145].

SI Table 2. Rate constants used for kinetic simulation^a.

	K_{eq}	k_{forward}	k_{reverse}
K_1	14×10^{-3}	557	8
K_2	0.62	0.45	0.28
K_3	500	$2 \cdot 10^{-3}$	1
K_4, K_7	2×10^{-5}	1×10^5	2
K_5, K_6	9×10^{-8}	1×10^6	0.09
K_8	NA	300	0
K_9	NA	Varied	0
K_{10}	NA	50	0

K_{11} NA Varied 0

^aThe units for equilibrium constants are molar for K_1, K_4 - K_7 or unitless for others. The units for rate constants are $M^{-1} s^{-1}$ or s^{-1} . The equilibrium constants of K_1, K_2, K_5 and K_7 as well as the rate constants for K_1 and K_2 are the experimentally determined values (see text).

The inhibition of k_{cat} seen in the experimental data was modeled either by slowing this step further (Conditions 1) or by slowing the chemistry step (Conditions 2). EC_{50} values were simulated at 0.5 mM and 100 mM glucose. The sensitivity of EC_{50} to the glucose concentration was calculated using the ratio of these two values, as shown in SI Table 3. The $K_{D, GKA}$ values were experimentally determined. Under the simulation condition 1, EC_{50} is more sensitive to the changes in glucose concentration as shown with a larger ratio (29 vs. 11), while condition 2 shows EC_{50} is less sensitive than $K_{D, GKA}$ (9 vs. 11). Although the EC_{50} values are also dependent on other kinetic parameters, these results confirm that activator effects on the rate of product release or chemistry can lead to different glucose dependencies for EC_{50} .

SI Table 3. Simulation results

	Condition 1	Condition 2
$k_9 (s^{-1})$	300	50
$k_{11} (s^{-1})$	25	50
EC_{50} ratio ^a	29	9
$K_{D, GKA}$ ratio ^b	11	11

^aThe EC_{50} ratio was calculated by dividend the simulated EC_{50} value at 0.5 mM glucose by the simulated value at 100 mM glucose. ^bThe $K_{D, GKA}$ ratio was calculated from the experimentally determined values of $K_{D, GKA}$ at 0.5 and 100 mM glucose shown in Figure 3.