Supplemental Material - Ortiz et al

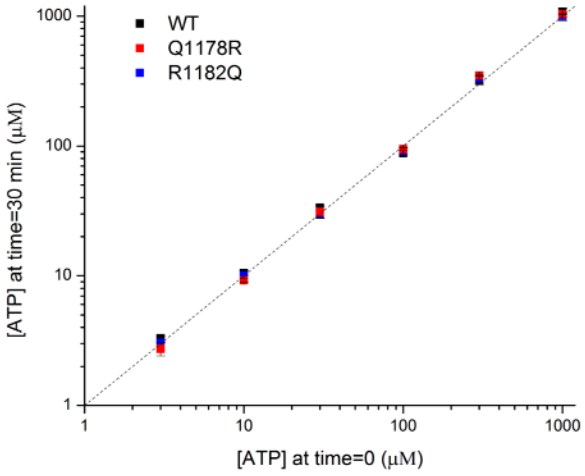
Mathematica (Wolfram Research, Champaign, IL) was used to do the algebraic derivations in this supplement. The *Mathematica* code is available upon request.

Luciferase Assay for ATP

ATP Reaction

Reaction conditions were similar to binding displacement experiments, however without [3H]GBC, and included an ATP-regenerating system consisting of 50 units/ml creatine phosphokinase (type I, from rabbit muscle, Sigma), 20mM creatine phosphate and 25mM MgCl2, and the indicated amounts of MgATP. 100µL aliquots were taken in triplicate before and after 30 minutes of incubation at 37°C and analyzed with a luciferase assay (Sigma), which indicated ATP levels remained stable in all membrane preps.

The results of determining ATP levels at time = 0 and after 30 minutes at 37°C with membranes present is shown in Fig. S1.



ADP Reaction

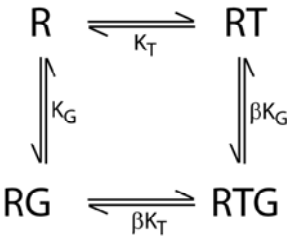
ADP (µM) at time = 0	ATP (µM) at time = 30 min
3	n.d.
10	n.d.
30	n.d.
100	n.d.
300	3.6 ± 0.1
1000	30.0 ± 0.3

Reactions included the indicated amounts of MgADP, 1mM free Mg^{2+}, and 10 mM AMP to inhibit endogenous adenylate kinases in membranes containing WT SUR1. Measurements were taken in triplicate before and after 30 minutes of incubation at 37°C as above, which indicated adenylate kinases were strongly inhibited up to 100 µM MgADP. Higher [MgADP] led to some ATP production, indicating steady-state conditions. The bioluminescent assay kit (Sigma Chemical Co.) for the detection of ATP was used in the functional range between 80 nM to 80 µM. Hence, non-detectable (n.d.) values were < 80 nM.

Allosteric Analysis

Estimating the linkage between GBC and ATP^{4-} *binding on SUR1: Four State Allosteric Model*

A 4-state model, shown below and in text Fig. 2B was used to describe the linkage between the binding of ATP^{4-} and [^{3}H]GBC on $SUR1_{Q1178R}$:



The model assumes that NBD1 of $SUR1_{Q1178R}$ (R) is fully occupied at concentrations well below the mM values of ATP^{4-} (T) needed to displace [^{3}H]GBC (G). The K's are the equilibrium dissociation constants of the receptor, R, for ATP^{4-} (T, K_T) at NBD2 and [^{3}H]GBC (G, K_G), respectively. β is the allosteric constant; if $\beta > 1$, the affinity of SUR1 for sulfonylureas is reduced by nucleotide binding. Following Wyman and Gill (1) the binding equation was derived from the binding partition function, the sum of the different species relative to one reference species, taken here as the unligated receptor, R. The partition function for the 4-state model is:

$$P = \frac{[R] + [RG] + [RT] + [RTG]}{[R]}$$

substituting the individual dissociation constants gives the binding polynomial:

$$P = 1 + \frac{[G]}{K_G} + \frac{[T]}{K_T} + \frac{[G][T]}{\beta K_G K_T}$$

$\overline{G}$, the amount of [^{3}H]GBC specifically bound per mole of SUR1, which is dependent on both [G] and $[ATP^{4-}]$, is given by:

$$\overline{G} = \frac{\partial \ln P}{\partial \ln G} = \frac{[G]}{P}\frac{\partial P}{\partial G}$$

$$\overline{G} = \frac{G\left(\frac{1}{Kg} + \frac{T}{Kg\,Kt\,\beta}\right)}{1 + \frac{G}{Kg} + \frac{T}{Kt} + \frac{G\,T}{Kg\,Kt\,\beta}}$$

The experimental variable plotted in the figures is Spec Bound [^{3}H]GBC $\equiv \frac{\overline{G}}{\overline{G}_{\text{no ATP}}}$

$$spBdGBC \equiv \frac{\left(1 + \frac{G}{Kg}\right) Kg\left(\frac{1}{Kg} + \frac{T}{Kg\,Kt\,\beta}\right)}{1 + \frac{G}{Kg} + \frac{T}{Kt} + \frac{G\,T}{Kg\,Kt\,\beta}}$$

A *Mathematica* function, NonlinearModelFit, was used to minimize least squares differences and estimate the best fit parameters for the equation spBDGBC to the data in text Fig. 2B for wildtype, Q1178R and R1182Q sulfonylurea receptors after constraining K_G to values based on the saturation data in text Table 1, and free [^{3}H]GBC = 0.00085, the free concentration of [^{3}H]GBC after subtracting 15% non-specific binding. All concentrations used are in μM.

The parameter results for wildtype, Q1178R and R1182Q SUR1 are given in Table S1. The curves plotted in Fig.2B use these parameters and the equation spBDGBC.

Estimating the linkage between [^{3}H]GBC and ATP^{4-} binding on SUR1$_{Q1178R}$ by a second method: K_{OBS} vs [ATP^{4-}]

The linkage between the [^{3}H]GBC and ATP^{4-} binding sites was estimated by a second method. The equation for $\overline{G}$,

$$\overline{G} = \frac{G\,(T+\beta\,Kt)}{\beta\,Kg\,(Kt+T)+G\,(T+\beta\,Kt)}$$

can be rearranged into a standard binding isotherm,

$$\overline{G} = \frac{[G]}{[G]+K_{OBS}}$$

where K_{OBS} depends on the concentration of ATP^{4-} and the allosteric constant, β.

$$K_{OBS} = \beta\,K_G\left(\frac{[T]+K_T}{[T]+\beta\,K_T}\right)$$

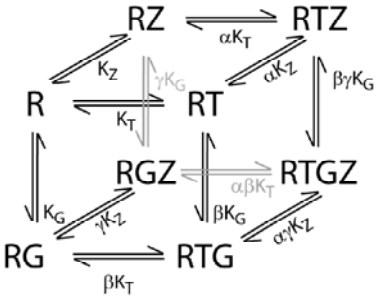
when [ATP^{4-}] = T = 0, $K_{obs} = K_G$. The values of β, K_G and Kt were estimated by fitting the Kobs equation to plots of Kobs versus [ATP^{4-}]. NonlinearModelFit, was used to minimize the least squares differences and estimate the best fit parameters for the nonlinear model, $K_{OBS} = \beta\,K_G\left(\frac{[T]+K_T}{[T]+\beta\,K_T}\right)$, with the constraint that K_G = 0.001. All concentrations are given in μM. The parameters are given in Table S1; the curve is plotted in Fig. 3.

SUR1	TABLE S1 β	K_T (μM)
WT	14 ± 10(0.2)	12200 ± 4030(0.02)
R1182Q	6.8 ± 0.9(<0.001)	1250 ± 258(0.003)
Q1178R	9.3 ± 1.8(<0.001)	1000 ± 179(<0.001)
Q1178R K_{OBS} method	10.5 ± 0.8(0.005)	846 ± 142(0.03)
	± SE (P values)	± SE (P values)

The estimated K_D for the binding of ATP^{4-} to wildtype SUR1 is >12 mM. The statistical significance of β is lower for this limited dataset. The fitting parameters for Q1178R derived from two approaches are in reasonable agreement.

Estimating the linkage between GBC, Diazoxide and ATP^{4-} binding on SUR1: Eight State Allosteric Model

The 8-state equilibrium model shown below, and in Fig. 6A, links the binding sites for diazoxide, GBC and ATP^{4-} in SUR1.



$\overline{G}_Z$, the amount of [^{3}H]GBC specifically bound per mole of SUR1, dependent on [Dz], [G] and [ATP^{4-}], was derived from the binding polynomial, PDz, as outlined above.

$$PDz = 1 + \frac{G}{Kg} + \frac{Dz}{Kz} + \frac{T}{Kt} + \frac{Dz\,T}{Kt\,Kz\,\alpha} + \frac{G\,T}{Kg\,Kt\,\beta} + \frac{Dz\,G}{Kg\,Kz\,\gamma} + \frac{Dz\,G\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}$$

$$\overline{Gz} = \left(G\left(\frac{1}{Kg} + \frac{T}{Kg\,Kt\,\beta} + \frac{Dz}{Kg\,Kz\,\gamma} + \frac{Dz\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}\right)\right) \Big/ \left(1 + \frac{G}{Kg} + \frac{Dz}{Kz} + \frac{T}{Kt} + \frac{Dz\,T}{Kt\,Kz\,\alpha} + \frac{G\,T}{Kg\,Kt\,\beta} + \frac{Dz\,G}{Kg\,Kz\,\gamma} + \frac{Dz\,G\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}\right)$$

To analyze the linkage between the binding sites for Dz, GBC and ATP^{4-} on $SUR1_{Q1178R}$, the changes in [^{3}H]GBC binding when one ligand was varied while the second was held constant were studied. The experimental variables, SpecBound [^{3}H]GBC (varying Dz) $\equiv \frac{\overline{G}z}{\overline{G}z(\text{noDz})}$ and Spec Bound [^{3}H]GBC (varying ATP) $\equiv \frac{\overline{G}z}{\overline{G}z(\text{noATP})}$, are given by:

$$\frac{\overline{G}z}{\overline{G}z\,(\text{noDz})} \equiv \left(\left(1 + \frac{G}{Kg} + \frac{T}{Kt} + \frac{G\,T}{Kg\,Kt\,\beta}\right)\left(\frac{1}{Kg} + \frac{T}{Kg\,Kt\,\beta} + \frac{Dz}{Kg\,Kz\,\gamma} + \frac{Dz\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}\right)\right) \Big/ \left(\left(\frac{1}{Kg} + \frac{T}{Kg\,Kt\,\beta}\right)\left(1 + \frac{G}{Kg} + \frac{Dz}{Kz} + \frac{T}{Kt} + \frac{Dz\,T}{Kt\,Kz\,\alpha} + \frac{G\,T}{Kg\,Kt\,\beta} + \frac{Dz\,G}{Kg\,Kz\,\gamma} + \frac{Dz\,G\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}\right)\right)$$

$$\frac{\overline{G}z}{\overline{G}z\,(\text{noATP})} \equiv \left(\left(1 + \frac{G}{Kg} + \frac{Dz}{Kz} + \frac{Dz\,G}{Kg\,Kz\,\gamma}\right)\left(\frac{1}{Kg} + \frac{T}{Kg\,Kt\,\beta} + \frac{Dz}{Kg\,Kz\,\gamma} + \frac{Dz\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}\right)\right) \Big/ \left(\left(\frac{1}{Kg} + \frac{Dz}{Kg\,Kz\,\gamma}\right)\left(1 + \frac{G}{Kg} + \frac{Dz}{Kz} + \frac{T}{Kt} + \frac{Dz\,T}{Kt\,Kz\,\alpha} + \frac{G\,T}{Kg\,Kt\,\beta} + \frac{Dz\,G}{Kg\,Kz\,\gamma} + \frac{Dz\,G\,T}{Kg\,Kt\,Kz\,\alpha\,\beta\,\gamma}\right)\right)$$

respectively. The binding parameters, α, γ and K_Z, which minimize the combined weighted sum of the squares of the differences between data points and predicted values for the two constrained models were determined using a *Mathematica* function, NMinimize. Based on the determinations above, the fitting was constrained using: $[ATP^{4-}] = 2000$, free [^{3}H]GBC = .00085, $\beta = 9.9$, $K_G = .001$ and $K_T = 925$. The β and K_T values represent the average of the values determined above. The curves in Fig. 5A and 5B use these parameters and the values for α, γ and Kz in Table S2.

	Table S2	
α	γ	K_Z (μM)
0.0082	1.3	6600.

References

(1) Jeffries Wyman and Stanley J. Gill (1990) *Binding and Linkage: Functional Chemistry of Biological Macromolecules,* University Science Books, Mill Valley, CA

(2) The *Mathematica* package "ErrorBarLogPlots" by Frank Rice was obtained from the Wolfram Library Archive, library.wolfram.com/infocenter/MathSource/6747