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online data

SUPPLEMENTARY ONLINE DATA

Mutational analysis of allosteric activation and inhibition of glucokinase

Bogumil ZELENT*, Stella ODILI*, Carol BUETTGER*, Dorothy K. ZELENT*, Pan CHEN†, Deborah FENNER‡, Joseph BASS‡, Charles STANLEY†, Monique LABERGE§¶, Jane M. VANDERKOOI*, Ramakanth SARABU‖, Joseph GRIMSBY‖ and Franz M. MATSCHINSKY*[1]

*Department of Biochemistry and Biophysics and Diabetes Research Center, School of Medicine, University of Pennsylvania, 415 Curie Blvd, 700 CRB, Philadelphia, PA 19104, U.S.A., †Division of Endocrinology, The Children's Hospital of Philadelphia, 34th Street and Civic Center Blvd., Philadelphia, PA 19104, U.S.A., ‡Department of Medicine, Feinberg School of Medicine, Northwestern University, Evanston, IL 60208, U.S.A., §Royal Military College St-Jean, St-Jean, QC J0J 1R0, Canada, ¶Concordia University, Department of Biology, Montréal, QC H3G 1M8, Canada, and ‖Departments of Discovery Chemistry and Metabolic Diseases, Hoffmann-La Roche, Nutley, NJ 07110, U.S.A.

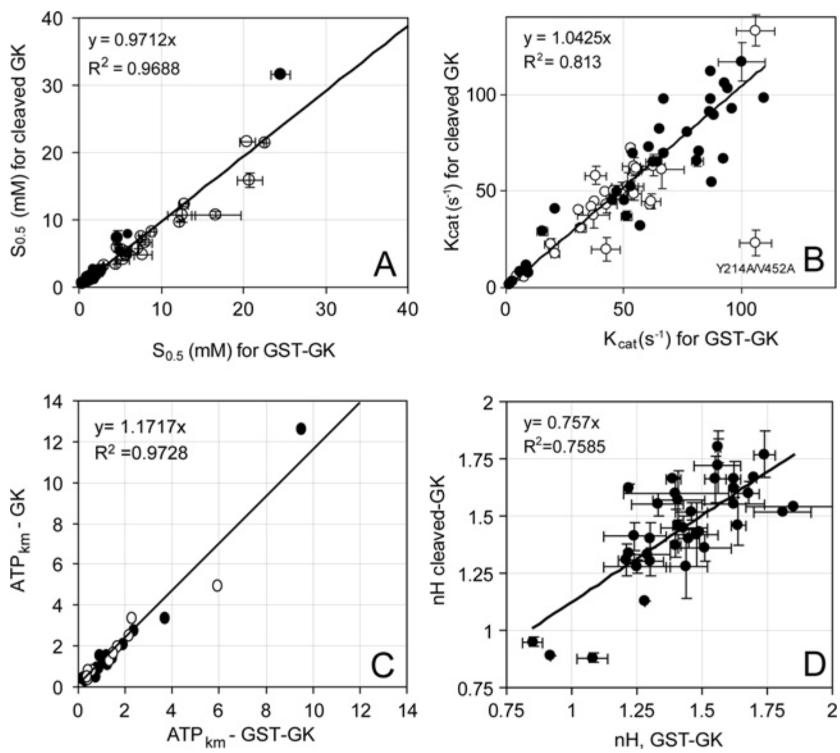

Figure S1 Comparison of GK enzyme kinetics

Comparison between GK wild-type (WT) and its mutants, and GST–GK–WT and its mutants for D-glucose $S_{0.5}$ (**A**), k_{cat} (**B**), $K_{m, ATP}$ (**C**) and h ('nH') (**D**) values. Results are taken from Table 1 of the main text and Supplementary Table S1 and are complemented by results (open circles) published previously [1]. Results are means ± S.E.M.

REFERENCE

1 Zelent, B., Odili, S., Buettger, C., Shiota, C., Grimstoy, J., Taub, R., Magnuson, M. A., Vanderkooi, J. M. and Matschinsky, F. M. (2008) Sugar binding to recombinant wild-type and mutant glucokinase monitored by kinetic measurement and tryptophan fluorescence. Biochem. J. **413**, 269–280

[1] To whom correspondence should be addressed (email matsch@mail.med.upenn.edu).

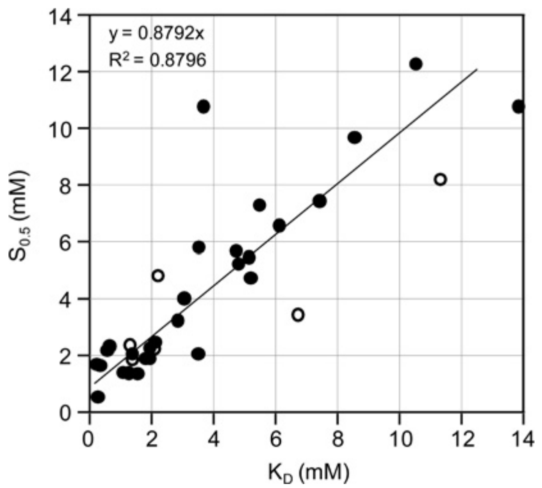

Figure S2 Comparison of the glucose $S_{0.5}$ for the phosphorylation reaction of the wild-type (WT) GK and mutants with the K_d for glucose binding measured by TF increase in the absence of enzymatic turnover

Results are taken from Tables 1 and 4 of the main text and are complemented by results (open circles) published previously [1].

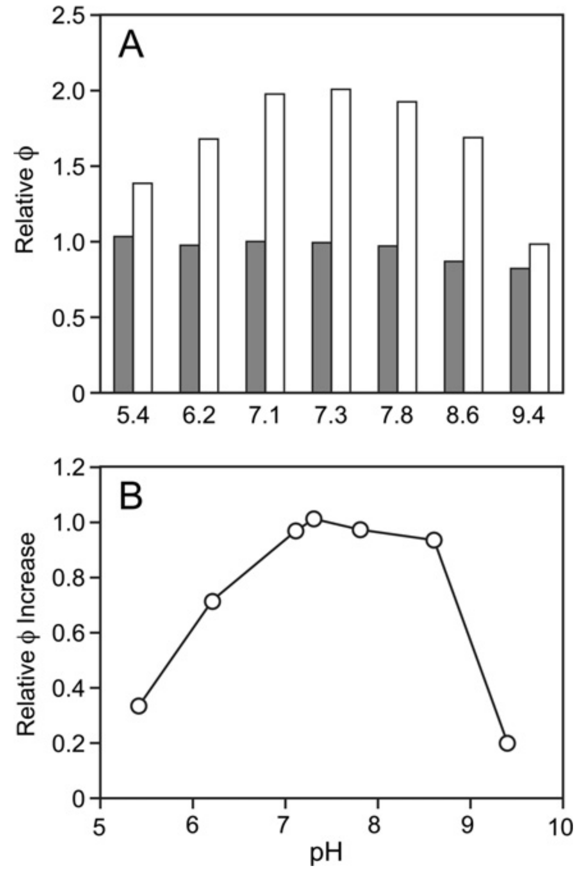

Figure S3 Effect of pH on relative quantum yield of TF of GK in the absence and presence of 300 mM glucose

(**A**) The results of TF measurements in the absence (closed bars) and presence (open bars) of saturating levels of glucose. (**B**) shows relative change of GK fluorescence over the pH range studied. Note that basal fluorescence is little affected by pH.

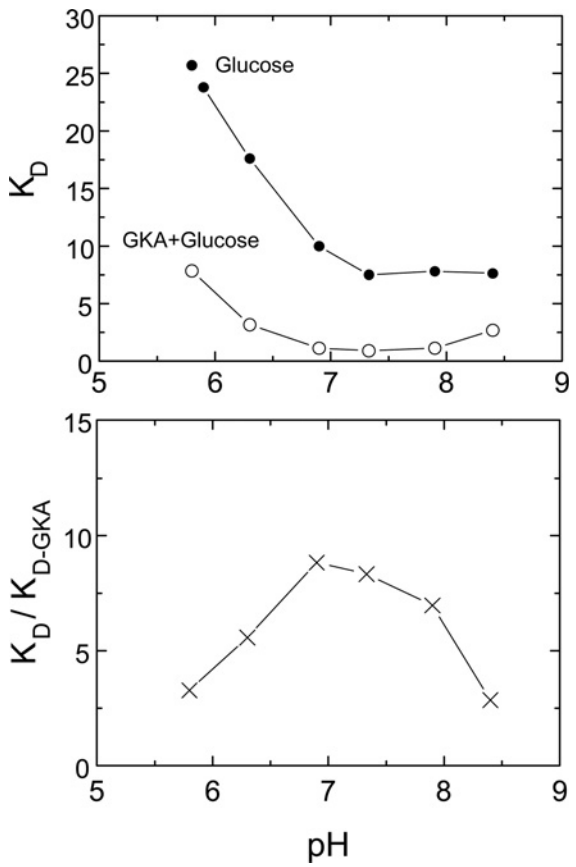

Figure S4 Effect of pH on binding constants

(**A**) pH effect on wild-type (WT) GK/glucose binding constants in the absence (closed circles) and presence (open circles) of 20 μM GKA. (**B**) the ratio of K_d values in the absence and presence of GKA over the pH range studied, i.e. the pH-dependency of the GKA effect is shown.

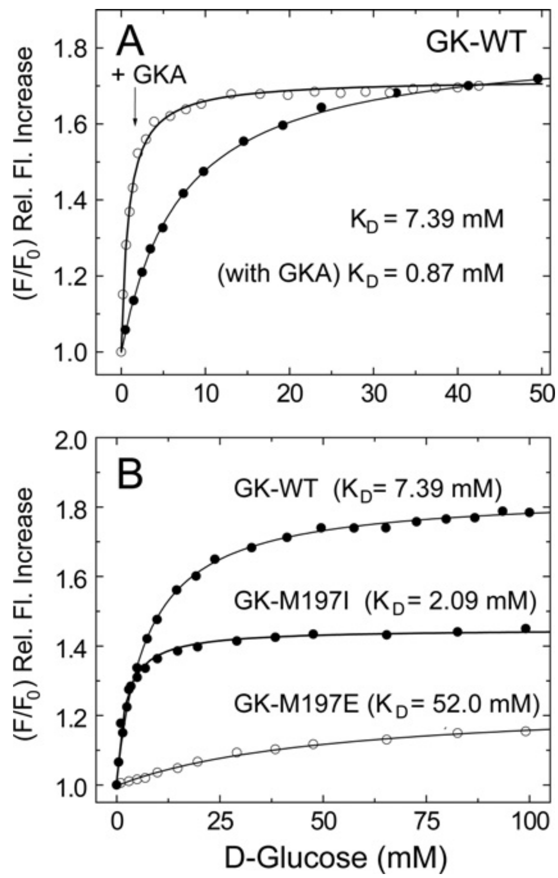

Figure S5 Glucose-dependent relative fluorescence increase for several enzymes

(**A**) Glucose binding to GK wild-type (WT) in the absence and presence of 20 μM GKA. (**B**) Glucose binding to GK-M197I and GK-M197E as compared with GK wild-type. The excitation wavelength was 280 nm and emission wavelength was 340 nm. The sample contained 5 mM phosphate (pH 7.3), 100 mM KCl, 1 mM DTT and 1 μM enzyme. Results were fitted to the Langmuir saturation function $F = F_0 + (F_{sat} - F_0)([\text{glucose}]/([\text{glucose}] + K_d)$.

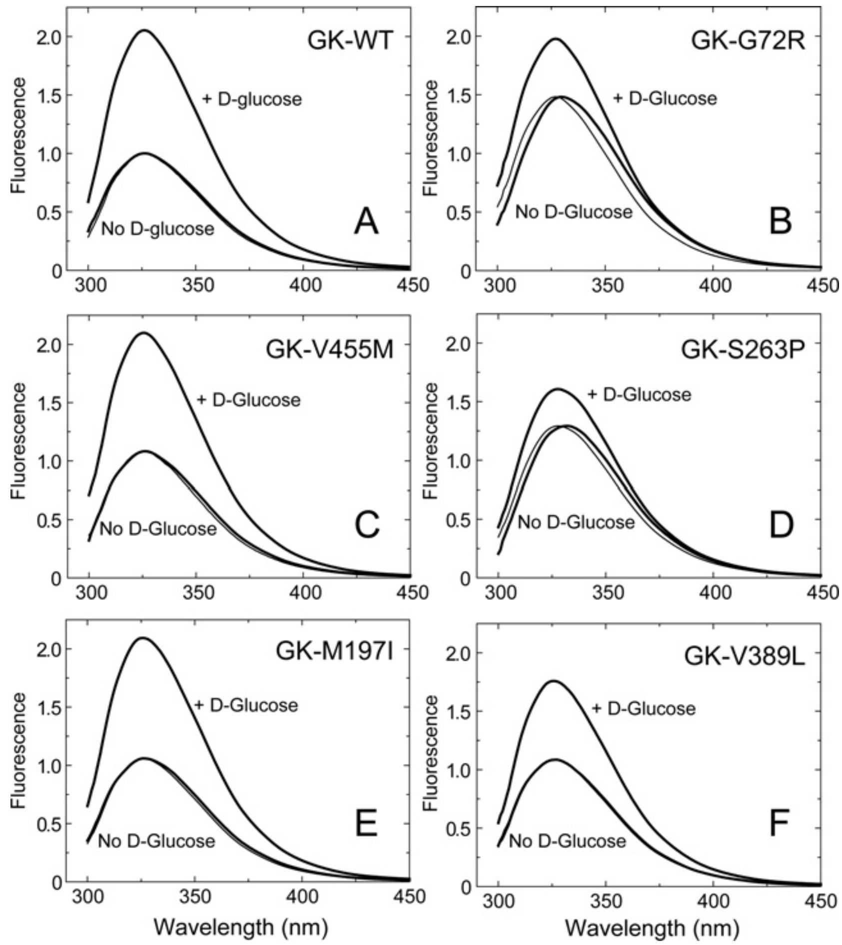

Figure S6 Fluorescence emission spectra of GK wild-type (WT) and mutant enzymes as indicated in the absence and presence of 300 mM D-glucose

Spectral blue shifts for G72R and S263P are indicated by relativizing the spectra. Spectral shifts are not observable for the wild-type enzyme nor for M197I, V389L and V455M. The excitation wavelength was 295 nm. The sample contained 5 mM phosphate (pH 7.3), 100 mM KCl, 1 mM DTT and 3 μM enzyme.

Table S1 Kinetic parameters of wild-type (WT) and mutant GST–GK fusion proteins stored for days to weeks in the presence of 50 mM D-glucose

Mutant	Yield (mg/l)	k_{cat} (s^{-1})	Glucose $S_{0.5}$ (mM)	h	$K_{m, ATP}$ (mM)	AI
WT ($n=5$)	43.4 ± 3.77	62.3 ± 5.00	7.55 ± 0.23	1.74 ± 0.04	0.41 ± 0.03	1.45 ± 0.11
V62M ($n=6$)	40.8 ± 8.97	51.3 ± 2.32	4.67 ± 0.21	1.44 ± 0.08	0.41 ± 0.05	4.52 ± 0.35
S64P ($n=2$)	27.9 (27.4, 28.6)	64.5 (58.5, 70.5)	2.46 (2.81, 2.11)	1.14 (0.962, 1.32)	0.3 (0.32, 0.28)	19.6 (17.4, 21.8)
S64Y ($n=2$)	27.8 (20.7, 34.9)	125 (92.4, 158)	1.95 (1.69, 2.2)	1.55 (1.64, 1.45)	1.47 (1.32, 1.62)	28.1 (23.6, 32.5)
T65I ($n=2$)	11.3 (13.5, 9.08)	19.2 (22.1, 16.3)	1.84 (1.92, 1.76)	1.30 (1.25, 1.35)	0.64 (0.73, 0.55)	5.91 (6.92, 4.90)
G68K ($n=2$)	62.6 (64.8, 60.4)	42.6 (48.2, 37.0)	2.60 (2.46, 2.73)	1.29 (1.38, 1.20)	0.26 (0.26, 0.25)	10.6 (11.8, 9.38)
G68V ($n=2$)	30.2 (31.2, 29.2)	45.1 (39, 51.2)	2.26 (2.33, 2.18)	1.34 (1.28, 1.4)	0.255 (0.25, 0.26)	13.6 (12.0, 15.1)
G72R ($n=5$)	23.7 ± 4.13	31.7 ± 2.13	5.32 ± 0.29	1.45 ± 0.04	0.76 ± 0.04	2.47 ± 0.24
V91L ($n=2$)	68.7 (72.3, 65.0)	54.8 (56.4, 53.1)	1.37 (1.48, 1.25)	1.48 (1.57, 1.39)	0.51 (0.54, 0.48)	28.2 (29.8, 26.6)
K140E ($n=4$)	31.6 ± 1.87	31.0 ± 1.60	12.5 ± 1.72	1.41 ± 0.10	0.24 ± 0.0.3	0.84 ± 0.11
M197E ($n=2$)	6.82 (6.86, 6.78)	21.0 (22.8, 19.2)	56.2 (58.3, 54.0)	1.30 (1.31, 1.29)	0.20 (0.21, 0.19)	0.11 (0.12, 0.10)
M197I ($n=2$)	62.1 (62.4, 61.8)	38.1 (42.6, 33.6)	2.55 (2.77, 2.33)	1.56 (1.57, 1.54)	1.46 (1.70, 1.22)	4.78 (5.02, 4.49)
M197I-A379T ($n=3$)	35.8 ± 3.16	45.8 ± 2.63	4.73 ± 0.30	1.51 ± 0.10	2.38 ± 0.15	2.55 ± 0.14
M197L ($n=2$)	34.5 (35.9, 33.0)	54.4 (59.2, 49.6)	5.35 (6.20, 4.49)	1.40 (1.60, 1.20)	1.03 (1.14, 0.91)	3.76 (3.97, 3.55)
C213R ($n=2$)	32.1 (31.6, 32.5)	48.9 (53.4, 44.4)	20.5 (21.4, 19.6)	1.49 (1.52, 1.46)	0.79 (0.81, 0.76)	0.87 (1.28, 0.46)
Y214A ($n=2$)	28.3 (30.2, 26.3)	100 (110, 90.1)	1.52 (1.70, 1.34)	1.24 (1.35, 1.12)	0.89 (0.92, 0.86)	50.0 (43.4, 56.5)
Y214A/V452A ($n=5$)	28.6 ± 2.60	106 ± 6.65	0.39 ± 0.06	1.08 ± 0.06	1.15 ± 0.06	250 ± 34.6
Y214C ($n=3$)	39.2 ± 4.85	62.7 ± 3.50	1.54 ± 0.19	1.33 ± 0.10	1.24 ± 0.03	21.7 ± 1.86
Y215A ($n=2$)	65.6 (65.8, 65.4)	37.8 (38.2, 37.4)	2.13 (2.14, 2.12)	1.41 (1.39, 1.43)	0.46 (0.47, 0.45)	10.9 (11.4, 10.3)
C252Y ($n=2$)	13.1 (15.2, 11.0)	15.5 (17.8, 13.2)	24.5 (25.7, 23.2)	1.85 (1.67, 2.03)	0.56 (0.56, 0.55)	0.06 (0.06, 0.05)
S263P ($n=2$)	40.6 (44.0, 37.1)	61.6 (57.6, 65.6)	12.3 (12.3, 12.2)	1.55 (1.59, 1.51)	0.57 (0.63, 0.51)	1.10 (1.13, 1.06)
M298K ($n=3$)	34.3 ± 3.28	37.2 ± 6.80	15.9 ± 3.05	1.25 ± 0.13	3.70 ± 0.25	0.76 ± 0.13
S336L ($n=2$)	16.3 (20.5, 12.0)	1.84 (2.70, 0.98)	7.81 (8.91, 6.71)	0.85 (0.89, 0.81)	9.50 (10.4, 8.60)	0.07 (0.09, 0.04)
A379T ($n=2$)	32.6 (34.0, 31.2)	66.0 (75.7, 56.2)	12.8 (13.3, 12.3)	1.68 (1.72, 1.64)	0.74 (0.75, 0.73)	0.67 (0.67, 0.66)
V389L ($n=2$)	20.1 (21.7, 18.8)	67.1 (68.6, 65.5)	3.45 (3.63, 3.26)	1,54 (1.49, 1.58)	0.753 (0.78, 0.73)	7.53 (7.44, 7.61)
K414E ($n=2$)	11.5 (13.4, 9.50)	42.4 (48.6, 36.2)	7.17 (7.10, 7.24)	1.81 (1.70, 1.91)	0.93 (1.04, 0.82)	1.24 (1.44, 1.04)
P417R ($n=4$)	7.17 ± 1.04	54.2 ± 3.19	8.05 ± 0.84	1.46 ± 0.06	1.91 ± 0.38	1.63 ± 0.15
E442K ($n=2$)	61.0 (63.5, 58.5)	51.6 (58.2, 44.9)	5.85 (6.12, 5.57)	1.56 (1.65, 1.46)	1.20 (1.20, 1.20)	1.50 (1.71, 1.29)
V452L ($n=2$)	27.8 (32.5, 23)	89.9 (90.2, 89.7)	3.16 (4.31, 2)	1.83 (2.16, 1.5)	0.43 (0.43, 0.43)	27.3 (30.8, 23.8)
454-Ala ($n=3$)	10.9 ± 3.05	53.2 ± 2.89	1.07 ± 0.04	1.22 ± 0.01	0.29 ± 0.04	39.5 ± 1.71
V455M ($n=2$)	29.9 (31.1, 28.7)	55.4 (57.8, 53.0)	3.02 (3.06, 2.97)	1.62 (1.60, 1.63)	0.37 (0.29, 0.45)	6.85 (7.14, 6.56)
A456V ($n=3$)	6.01 ± 1.27	81.3 ± 2.60	2.03 ± 0.11	1.40 ± 0.02	0.25 ± 0.004	24.0 ± 2.18

Table S2 Effect of GKA on recombinant human wild-type (WT) and mutant GKs expressed as GST–GK fusion proteins

A denotes the maximal fold drug effect on the k_{cat} value, *B* on the glucose affinity and *C* on the activity index. EC_{50} values are half maximal drug concentrations for the effect on the k_{cat}, the glucose affinity and the activity index. n.a., not applicable.

GST–GK	*A* (fold↑)	EC_{50} (μM^{A})	*B* (fold↓)	EC_{50} (μM^{B})	*C* (fold↑)	EC_{50} (μM^{C})	n^{ctr}/n^{act}	$^{ctr}K_{m, ATP}/^{act}K_{m, ATP}$
WT (*n* = 5)	1.51 ± 0.05	0.73 ± 0.13	4.27 ± 0.06	0.87 ± 0.06	19.6 ± 0.90	6.73 ± 0.46	1.13 ± 0.02	0.91 ± 0.05
V62M (*n* = 6)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.05 ± 0.06	1.15 ± 0.15
S64P (*n* = 2)	n.a., 0.91	n.a., 3.55	1.37 (1.52, 1.22)	21.7 (23.2, 20.3)	n.a., 1.38	n.a., 5.56	1.11 (1.05, 1.18)	1.36 (1.14, 1.57)
S64Y (*n* = 2)	1.08, n.a.	0.49, n.a.	1.9 (1.87,1.92)	1.23 (0.58, 1.88)	3.06 (3.17, 2.95)	2.89 (2.46, 3.31)	1.15 (1.05, 1.24)	1.35 (1.33, 1.37)
T65I (*n* = 2)	2.36 (2.44, 2.27)	5.79 (6.24, 5.34)	1.74 (1.69, 1.78)	3.49 (3.86, 3.12)	4.47 (4.26, 4.67)	7.38 (7.71, 7.05)	0.97 (1.00, 0.93)	1.10 (1.17, 1.02)
G68V (*n* = 2)	2.22 (2.31, 2.13)	1.33 (1.72, 0.94)	3.21 (2.97, 3.44)	0.93 (0.6, 1.27)	9.59 (8.4, 10.8)	4.89 (2.99, 6.79)	1.02 (0.99, 1.05)	0.45 (0.41, 0.49)
G68K (*n* = 2)	2.25 (2.34, 2.15)	3.79 (4.56, 3.01)	4.09 (4.15, 4.03	1.61 (2.06, 1.15)	11.2 (12.2, 10.2)	6.16 (7.06, 5.25)	1.30 (1.33, 1.26)	0.58 (0.62, 0.53)
G72R (*n* = 5)	1.59 ± 0.05	4.13 ± 0.90	1.86 ± 0.03	3.02 ± 0.62	2.92 ± 0.10	9.02 ± 1.12	1.03 ± 0.04	1.16 ± 0.04
V91L (*n* = 2)	1.59 (1.63, 1.55)	3.75 (4.52, 2.97)	2.83 (2.87, 2.78)	4.85 (6.37, 3.32)	5.96 (6.07, 5.85)	6.94 (7.04, 6.83)	1.21 (1.22, 1.20)	0.83 (0.86, 0.80)
K140E (*n* = 4)	1.74 ± 0.06	1.24 ± 0.30	5.89 ± 0.31	1.47 ± 0.37	19.1 ± 3.20	11.7 ± 2.33	0.95 ± 0.03	0.67 ± 0.20
M197I (*n* = 2)	1.76 (1.85, 1.67)	1.79 (2.26, 1.31)	3.96 (4.27, 3.65)	0.51 (0.69, 0.32)	13.1 (13.3, 12.9)	4.46 (4.90, 4.02)	1.23 (1.25, 1.20)	1.27 (1.28, 1.26)
M197I-A379T (*n* = 3)	1.93 ± 0.08	0.89 ± 0.16	4.41 ± 0.20	0.78 ± 0.01	18.1 ± 0.80	4.93 ± 0.26	1.10 ± 0.09	1.28 ± 0.06
M197L (*n* = 2)	1.71 (1.71, 1.70)	0.61 (0.77, 0.44)	4.54 (4.24, 4.65)	0.71 (0.83, 0.58)	14.6 (14.2, 14.9)	5.42 (6.59, 4.25)	0.92 (0.78, 1.06)	1.30 (1.14, 1.45)
C213R (*n* = 2)	1.32 (1.34, 1.29)	1.21 (1.81, 0.60)	4.16 (4.19, 4.13)	1.23 (1.27, 1.19)	5.51 (8.70, 2.32)	7.28 (7.68, 6.88)	0.91 (0.94, 0.88)	1.41 (1.44, 1.38)
Y214A (*n* = 2)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.92 (0.94, 0.90)	0.99 (0.92, 1.06)
Y214A/V452A (*n* = 5)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.97 ± 0.09	0.96 ± 0.04
Y214C (*n* = 3)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.14 (1.22, 1.06)	1.02 (0.97, 1.06)
Y215A (*n* = 2)	1.73 (1.75, 1.70)	1.80 (1.85, 1.75)	3.22 (3.42, 3.02)	0.92 (1.17, 0.67)	8.21 (8.65, 7.77)	5.98 (5.11, 6.85)	1.09 (1.07, 1.11)	0.85 (0.91, 0.79)
C252Y (*n* = 2)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.20 (1.10, 1.30)	1.12 (1.10, 1.13)
S263P (*n* = 2)	1.50 (1.52, 1.47)	0.67 (0.83, 0.50)	5.51 (5.57, 5.45)	0.56 (0.60, 0.52)	19.3 (18.1, 20.4)	8.32 (7.08, 9.55)	1.11 (1.09, 1.12)	1.11 (1.05, 1.17)
M298K (*n* = 3)	1.27 ± 0.07	0.81 ± 0.22	8.78 ± 0.62	0.69 ± 0.11	10.9 ± 0.65	8.52 ± 0.61	1.12 ± 0.13	0.87 ± 0.03
S336L (*n* = 2)	1.36 (1.38, 1.33)	1.96 (2.63, 1.29)	22.7 (28.2, 17.2)	0.55 (0.86, 0.23)	8.10 (6.67, 9.52)	6.04 (7.00, 4.99)	1.04 (0.94, 1.13)	0.78 (0.81, 0.74)
A379T (*n* = 2)	1.66 (1.66, 1.65)	2.57 (2.72, 2.41)	4.58 (4.59, 4.57)	3.54 (4.51, 2.56)	29.8 (32.0, 27.6)	15.8 (19.5, 12.0)	1.11 (1.12, 1.10)	1.58 (2.23, 0.92)
V389L (*n* = 2)	1.37 (1.47, 1.27)	1.67 (0.84, 2.5)	3.3 (3.01, 3.59)	2.51 (1.92, 3.1)	9.49 (9.8, 9.18)	7.33 (5.77, 8.89)	1.08 (1.18, 0.98)	1.23 (1.57, 0.88)
K414E (*n* = 2)	1.35 (1.37, 1.32)	0.77 (0.60, 0.93)	5.39 (5.32, 5.46)	0.89 (0.96, 0.82)	18.0 (16.1, 19.8)	6.85 (6.38, 7.32)	1.20 (1.20, 1.19)	0.79 (0.83, 0.74)
P417R (*n* = 4)	1.51 ± 0.06	0.73 ± 0.26	4.78 ± 0.30	2.16 ± 0.57	14.2 ± 0.83	10.7 ± 1.78	0.96 ± 0.06	0.94 ± 0.13
E442K (*n* = 2)	1.68 (1.72, 1.63)	1.35 (1.74, 0.95)	3.98 (3.72, 4.24)	1.88 (1.61, 2.15)	24.4 (25.0, 23.8)	9.15 (9.47, 8.83)	1.03 (1.09, 0.96)	1.13 (1.27, 0.98)
V452L (*n* = 2)	n.a., 1.43	n.a., 3.53	2.8 (2.9, 2.69)	2.32 (2.37, 2.28)	5.44 (5.00, 5.89)	5.58 (5.61, 5.56)	1.17 (1.15, 1.20)	1.1 (1.11, 1.08)
454-Ala (*n* = 3)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.09 ± 0.04	1.09 ± 0.02
V455M (*n* = 2)	1.39 (1.39, 1.39)	5.97 (4.68, 7.26)	1.71 (1.71, 1.70)	4.45 (4.79, 4.10)	3.70 (3.95, 3.44)	10.7 (11.1, 10.3)	1.08 (1.09, 1.07)	1.13 (1.21, 1.04)
A456V (*n* = 3)	n.a.	n.a.	3.46 ± 0.22	1.20 ± 0.49	5.18 ± 0.57	5.36 ± 1.61	1.27 ± 0.02	1.56 ± 0.11

Table S3 pH-dependence of TF quantum yields for wild-type GK

The temperature was 20 °C and the excitation wavelength was 295 nm.

pH	$\phi_{average}$ no glucose	$\phi_{average}$ with glucose	Fluorescence increase (%)	Emission wavelength max no glucose (nm)	Emission wavelength with glucose (nm)
5.4	0.098	0.130	32.65	327	329
6.2	0.092	0.158	71.74	325	325
7.1	0.095	0.186	95.79	325	326
7.3	0.094	0.187	98.94	326	326
7.8	0.101	0.181	96.74	326	328
8.6	0.082	0.159	93.90	326	331
9.4	0.078	0.093	19.23	331	336

Table S4 Binding constants in mM for D-glucose using GK wild-type

Effect of pH on glucose binding in the absence and presence of 20 μM GKA measured by TF (25 °C, $\lambda_{ex} = 295$ nm and $\lambda_{em} = 340$ nm)

pH	$K_{d(D\text{-glucose})}$	$K_{d(GKA)}$	$K_{d(D\text{-glucose})}/K_{d(GKA)}$
5.8	25.70	7.84	3.28
5.9	23.79	–	–
6.3	17.60	3.16	5.57
6.9	9.98	1.13	8.83
7.3	7.50	0.87	8.33
7.9	7.80	1.12	6.96
8.4	7.63	2.68	2.85

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