## Supplemental material 1

In order to estimate the initial concentrations of G proteins which would be further utilized for kinetic simulations we assumed that GIRK-Gα-Gβγ system reaches steady-state after application of the agonist and there is complete dissociation of Gα from Gβγ . For “graded contribution” model steady-state concentrations of GIRK and Gβγ were estimated utilizing Eq. 3-10

$$Eq.3: C\_{0}+C\_{1}+C\_{2}+C\_{3}+C\_{4}=C\_{total} $$

$$Eq.4: C\_{1}=\frac{4∙C\_{0}∙Gβγ}{K\_{D}}$$

$$Eq.5: C\_{2}=\frac{3∙C\_{1}∙Gβγ}{2∙K\_{D}}$$

$$Eq.6: C\_{3}=\frac{2∙C\_{2}∙Gβγ}{3∙K\_{D}}$$

$$Eq.7: C\_{4}=\frac{C\_{3}∙Gβγ}{4∙K\_{D}}$$

$$Eq.8: Gβγ+C\_{1}+C\_{2}+C\_{3}+C\_{4}=Gβγ\_{total }$$

$$Eq.9: (f\_{1}∙C\_{1}+f\_{2}∙C\_{2}+f\_{3}∙C\_{3}+f\_{4}∙C\_{4})∙cf=I\_{total}$$

Eq.10 : cf = w$∙S∙A∙i$single$∙P$o,max

where Co-C4 are concentrations of GIRK channels bound to 0-4 Gβγ subunits, Ctotal is the total channel concentration, Gβγtotal is the total Gβγ concentration, f1-f4 are the contributions of C1-C4 states of channel occupancy to maximal open probability, cf is a conversion factor and I­total is the current recorded on application of agonist, W is the width of interaction space (10 nm), S is the oocyte membrane area (2$∙$ 107 µm2), Po,max is the maximal open probability under saturating Gβγ conditions (0.105) and isingle is the amplitude of unitary current (~ 0.6 pA in 24 meq K+ ) and A iss the Avogadro number (Yakubovich et al., 2015). Based on estimation of Gβγtotal, we subsequently calculated the Gαtotal value combining Eq. 3-10 with Eq. 11-13

$$Eq.11: GaGDPGβγ+Gβγ+CGβγ\_{1}+CGβγ\_{2}+CGβγ\_{3}+CGβγ\_{4}=Gβγ\_{total }$$

$$Eq.12: GaGDPGβγ+Ga=Ga\_{total }$$

$$Eq.13: (f\_{1}∙C\_{1}+f\_{2}∙C\_{2}+f\_{3}∙C\_{3}+f\_{4}∙C\_{4})∙cf=I\_{basal}$$

where Ibasal is the current recorded in the absence of agonist. Parenthetically, assuming a finite but very low affinity of GαGTP to Gβγ, 15 µM (which we utilized for time-course simulation of agonist activation) does not significantly change the results.

A similar way to estimate initial concentrations was implemented for Touhara et al. model. Eq. 4- 9, and 13 were respectively modified to account for cooperative binding of Gβγ to GIRK rendering:

$$Eq.14: C\_{1}=\frac{4∙C\_{0}∙Gβγ}{K\_{D}}$$

$$Eq.15: C\_{2}=\frac{3∙C\_{1}∙Gβγ}{2∙µ∙K\_{D}}$$

$$Eq.16: C\_{3}=\frac{2∙C\_{2}∙Gβγ}{3∙µ^{2}∙K\_{D}}$$

$$Eq.17: C\_{4}=\frac{C\_{1}∙Gβγ}{4∙µ^{3}∙K\_{D}}$$

$$Eq.18: C\_{4}∙cf=I$$

where I is respectively Itotal or Ibasal for calculations of Gβγtotal and Gαtotal.

## Supplemental material 2

For simulation of time-course of GIRK1/2 activation we generated a system of ordinary differential equation based on scheme of G-protein cycle and scheme of channel gating (Fig.4A and B).

Eq. 19: d(R)/dt =-R∙( k1f∙A+k2f∙Ggdp) +k1b∙RA+k2b∙RGGDP

Eq.20: d(RGGDP)/dt =-RGGDP∙(k3f∙A+k2b)+k3b∙RAGGDP+k2f∙R∙GGDP

Eq.21: d(RAGGDP)/dt = -RAGGDP∙(k3b+k5f+k4b)+k4f∙RA∙GGDP+k5b∙RAG0∙GDP+k3f∙RGGDP∙A

Eq.22: d(RA)/dt = -RA∙(k1b+k4f∙GGDP+k8b∙GαGDP)+k1f∙R∙A+k4b∙RAGGDP+k8f∙RAGαGDP

Eq.23: d(RAG0)/dt =-RAG0∙(k5b∙GDP+k6f∙GTP)+k5f∙RAGGDP +k6b∙RAGGTP

Eq.24: d(RAGGTP)/dt = - RAGGTP∙(k6b+k7f)+k6f∙RAG0∙GTP+k7b∙RAGαGTP∙Gβγ

Eq.25: d(RAGαGTP)/dt = -RAGαGTP∙(k8f+k7b∙Gβγ)+k7f∙RAGGTP+k8b∙RA∙GαGTP

Eq.26: d(GαGTP)/dt = - GαGTP ∙(k9f+k8∙RA)+k8f∙RAGαGTP

Eq.27: d(GαGDP)/dt = -k10f∙GαGDP∙Gβγ+k9f∙GαGDP

Eq.28: d(GGDP)/dt =-GGDP∙(k2f∙R+k4f∙RA)+k10f∙GαGDP∙Gβγ+k2b∙RGGDP+k4b∙RAGGDP

Eq.29: d(Gβγ)/dt =-Gβγ∙(k10f∙GαGDP+k7b∙RAGαGTP+4∙kon∙C0+3∙kon∙C1+2∙ kon∙C2+ kon∙C3)+k7f∙RAGGTP+k10b∙GGDP

+koff∙(C1+2∙C2+3∙C3+4∙C4)

Eq.30: d(C0)/dt = - 4∙kon∙C0∙Gβγ +koff∙C1

Eq.31: d(C1)/dt = -C1∙(3∙kon∙Gβγ+koff)+4∙ kon ∙C0∙Gβγ+2∙koff∙C2

Eq.32: d(C2)/dt = -C2∙(2∙kon∙Gβγ+2∙koff)+3∙kon∙C1∙Gβγ+3∙koff∙C3

Eq.33: d(C3)/dt = -C3∙(kon∙Gβγ+3∙koff)+2∙ kon∙C2∙Gβγ+4∙koff∙C4

Eq.34: d(C4)/dt = -4∙ koff ∙C4 +kon∙C3∙Gβγ

For all kinetic simulations GDP and GTP were kept constant and respectively 10 and 100 µM (Traut, 1994). A – agonist concentration – in our case ACh was assumed to step increase from 0 to 10 µM and also was kept constant.