γ-2 and GSG1L bind with comparable affinities to the tetrameric GluA1 core

Chenlu Yu, Hendrik F P Runge, Antara Mukhopadhyay, Gerd Zolles, Maximilian H Ulbrich

Supplementary Material

Supplementary Note 1

To model the binding of γ-2 and GSG1L to GluA1, we assume that the densities are in an equilibrium for all assemblies. From Fig. 5A we see:

$$A + G \underset{k_{Goff}}{\overset{4\,k_{Gon}}{\rightleftharpoons}} AG1$$

$$AG1 + G \underset{2\,k_{Goff}}{\overset{3\,k_{Gon}}{\rightleftharpoons}} AG2$$

etc. Then

$$[AG1] = 4\,K_G\,[A][G]$$

$$[AG2] = 3/2\,K_G\,[AG1][G] = 6\,{K_G}^2\,[A][G]^2$$

$$[AG3] = 2/3\,K_G\,[AG2][G] = 4\,{K_G}^3\,[A][G]^3$$

$$[AG4] = 1/4\,K_G\,[AG3][G] = {K_G}^4\,[A][G]^4$$

$$[A\gamma1] = 4\,K_\gamma\,[A][\gamma]$$

$$[A\gamma2] = 3/2\,K_\gamma\,[A\gamma1][\gamma] = 6\,{K_\gamma}^2\,[A][\gamma]^2$$

$$[A\gamma3] = 2/3\,K_\gamma\,[A\gamma2][\gamma] = 4\,{K_\gamma}^3\,[A][\gamma]^3$$

$$[A\gamma4] = 1/4\,K_\gamma\,[A\gamma3][\gamma] = {K_\gamma}^4\,[A][\gamma]^4$$

$$[AG1\gamma1] = 3\,K_\gamma\,[AG1][\gamma] = 12\,K_G\,K_\gamma\,[A][G][\gamma]$$

$$[AG1\gamma2] = K_\gamma\,[AG1\gamma1][\gamma] = 12\,K_G\,{K_\gamma}^2\,[A][G][\gamma]^2$$

$$[AG1\gamma3] = 1/3\;K_\gamma\,[AG1\gamma1][\gamma] = 4\,K_G\,{K_\gamma}^3\,[A][G][\gamma]^3$$

$$[AG2\gamma1] = 2\,K_\gamma\,[AG2][\gamma] = 12\,{K_G}^2\,K_\gamma\,[A][G]^2[\gamma]$$

$$[AG2\gamma2] = 1/2\;K_\gamma\,[AG2\gamma1][\gamma] = 6\,{K_G}^2\,{K_\gamma}^2\,[A][G]^2[\gamma]^2$$

$$[AG3\gamma1] = K_\gamma\,[AG3][\gamma] = 4\,{K_G}^3\,K_\gamma\,[A][G]^3[\gamma]$$

Because not all fluorescent protein molecules are functional, the bleaching steps counts do not equal the actual numbers of auxiliary subunits. We defined the probability of mNeonGreen to be functional as p and of mCherry as q. Since the receptors contained four GluA1-GFP(Y66L) and were usually labeled with multiple A647-Nb, we approximated that all receptor cores were visible (see Fig. 3C). Therefore, we could write the concentration of counted spots as:

$$[G_{count}] = [G]\,p$$

$$[\gamma_{count}] = [\gamma]\,q$$

$$[A_{count}] = [A] + [AG1]\,(1-p) + [AG2]\,(1-p)^2 + [AG3]\,(1-p)^3 + [AG4]\cdot(1-p)^4 + [A\gamma1]\,(1-q) + [A\gamma2]\,(1-q)^2 + [A\gamma3]\,(1-q)^3 + [A\gamma4]\,(1-q)^4 + [AG1\gamma1]\,(1-p)\,(1-q) + [AG2\gamma1]\,(1-p)^2\,(1-q) + [AG3\gamma1]\,(1-p)^3\,(1-q) + [AG1\gamma2]\,(1-p)\,(1-q)^2 + [AG2\gamma2]\,(1-p)^2\,(1-q)^2 + [AG1\gamma3]\,(1-p)\,(1-q)^3$$

$$[AG1_{count}] = [AG1]\,p + 2\,[AG2]\,p\,(1-p) + 3\,[AG3]\,p\,(1-p)^2 + 4\,[AG4]\,p\,(1-p)^3 + [AG1\gamma1]\,p\,(1-q) + 2\,[AG2\gamma1]\,p\,(1-p)\,(1-q) + 3\,[AG3\gamma1]\,p\,(1-p)^2\,(1-q) + [AG1\gamma2]\,p\,(1-q)^2 + 2\,[AG2\gamma2]\,p\,(1-p)\,(1-q)^2 + [AG1\gamma3]\,p\,(1-q)^3$$

$$[AG2_{count}] = [AG2]\,p^2 + 3\,[AG3]\,p^2\,(1-p) + 6\,[AG4]\,p^2\,(1-p)^2 + [AG2\gamma1]\,p^2\,(1-q) + 3\,[AG3\gamma1]\,p^2\,(1-p)\,(1-q) + [AG2\gamma2]\,p^2\,(1-q)^2$$

$$[AG3_{count}] = [AG3]\,p^3 + 4\,[AG4]\,p^3\,(1-p) + [AG3\gamma1]\,p^3\,(1-q)$$

$$[AG4_{count}] = [AG4]\,p^4$$

$$[A\gamma_{count}] = [A\gamma1]\,q + [A\gamma2]\,(1-(1-q)^2) + [A\gamma3]\,(1-(1-q)^3) + [A\gamma4]\,(1-(1-q)^4) + [AG1\gamma1]\,(1-p)\,q + [AG2\gamma1]\,(1-p)^2\,q + [AG3\gamma1]\,(1-p)^3\,q + [AG1\gamma2]\,(1-p)\,(1-(1-q)^2) + [AG2\gamma2]\,(1-p)^2\,(1-(1-q)^2) + [AG1\gamma3]\,(1-p)\,(1-(1-q)^3)$$

$$[AG1\gamma_{count}] = [AG1\gamma1]\,p\,q + 2\,[AG2\gamma1]\,p\,(1-p)\,q + 3[AG3\gamma1]\,p\,(1-p)^2\,q + [AG1\gamma2]\,p\,(1-(1-q)^2) + 2\,[AG2\gamma2]\,p\,(1-p)\,(1-(1-q)^2) + [AG1\gamma3]\,p\,(1-(1-q)^3)$$

$$[AG2\gamma_{count}] = [AG2\gamma1]\,p^2\,q + 3\,[AG3\gamma1]\,p^2\,(1-p)\,q + [AG2\gamma2]\,p^2\,(1-(1-q)^2)$$

$$[AG3\gamma_{count}] = [AG3\gamma1]\,p^3\,q$$

Because we could not distinguish 1, 2, 3 or 4 subunits of γ-2-mCherry, we used:

$$[A\gamma_{count}] = [A\gamma1_{count}] + [A\gamma2_{count}] + [A\gamma3_{count}] + [A\gamma4_{count}]$$

$$[AG1\gamma_{count}] = [AG1\gamma1_{count}] + [AG1\gamma2_{count}] + [AG1\gamma3_{count}]$$

$$[AG2\gamma_{count}] = [AG2\gamma1_{count}] + [AG2\gamma2_{count}]$$

$$[AG3\gamma_{count}] = [AG3\gamma1_{count}]$$

With the above equations and the counted number of protein complexes, we could fit the K_G and K_γ by least-square fitting. In practice, p was set to 0.77, which was fitted from GluA1-mNeonGreen experiments, and q was set to 0.65, which was our estimate from previous results and was also determined independently [Prangsma 2020].

Supplementary Note 2

Full nanobody sequence:

MKYLLPTAAAGLLLLAAQPAMAMDKSKSGKSGKGSDPQVQLVESGGALVQPGGSLRLSCAAS
GFPVNRYSMRWYRQAPGKEREWVAGMSSAGDRSSYEDSVKGRFTISRDDARNTVYLQMNSL
KPEDTAVYYCNVNVGFEYWGQGTQVTVSSKLAAALEHHHHHH

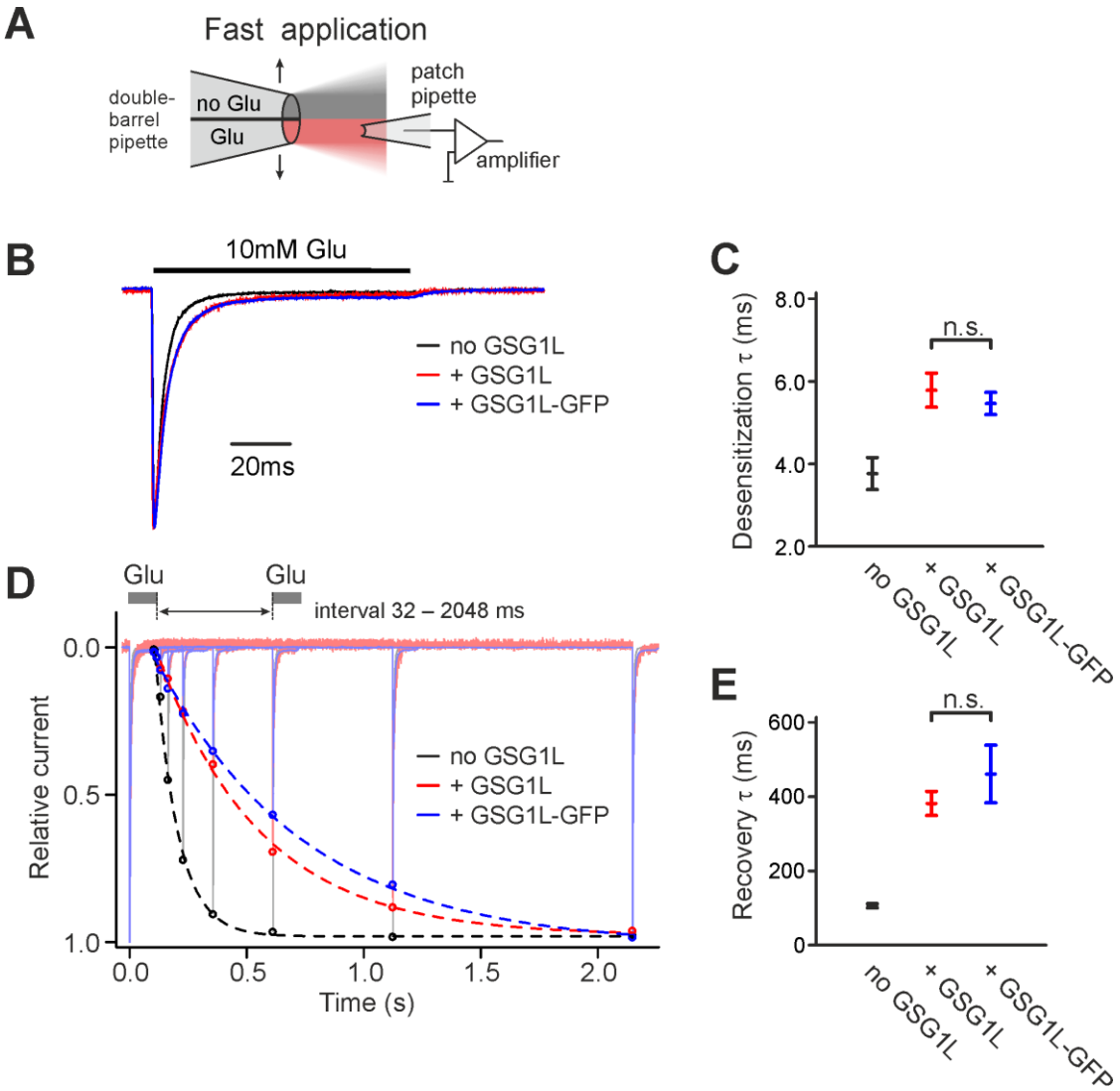
Green: PelB leader sequence

Orange: 4x lysine tag for labeling

Blue: anti-GFP nanobody

Red: linker and 6x His tag

Supplementary Figure 1



Supplementary Figure 1: GFP-labeled GSG1L is functional. (A) Current responses from outside-out patches were elicited by rapid application/removal of glutamate using a piezo-controlled fast application system with a double-barrel application pipette. (B) Representative current responses of AMPARs recorded upon 100 ms applications of 10 mM glutamate (black bar above current traces) from GluA1+GluA2 alone (black) or in combination with either GSG1L (red) or GSG1L-GFP (blue). (C) Time constants of desensitization for GluA1+GluA2 alone or with either GSG1L or GSG1L-GFP (n=4–8; mean±s.e.m.). GSG1L-GFP slows down the desensitization like untagged GSG1L. (D) Recovery of AMPARs with composition as in (B) from steady-state desensitization recorded with a double-pulse protocol (pair of two 100 ms glutamate pulse separated by increasing time intervals). Circles indicate peak currents recorded during the second pulse. Dashed lines are mono-exponential fits to the time course of the peak currents. (E) Recovery time constants (n=4–8; mean±s.e.m.). GSG1L-GFP slows down the recovery from desensitization like untagged GSG1L.