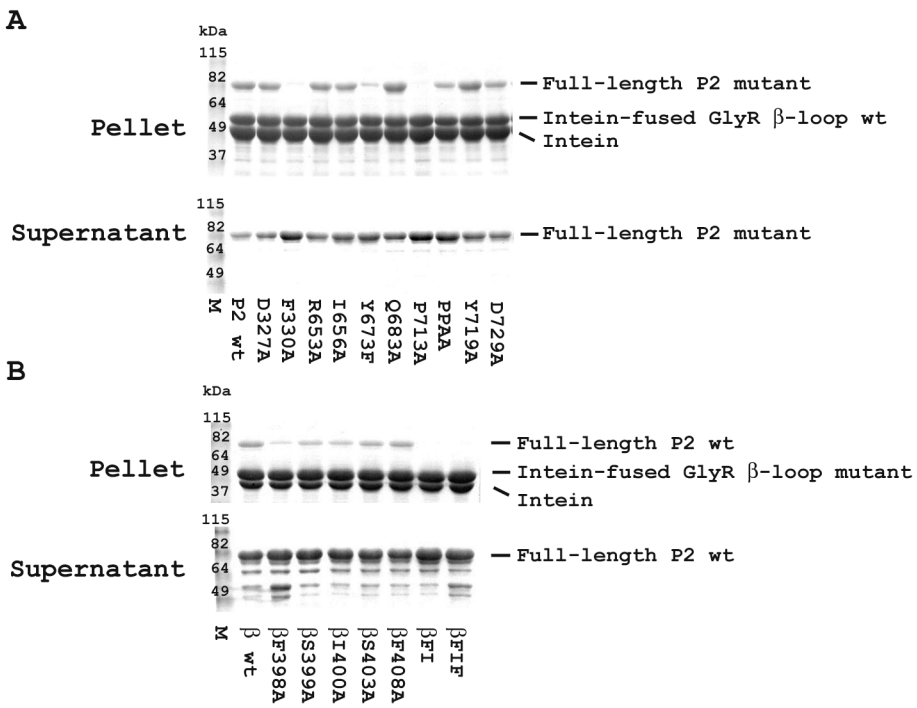

Supplementary Figure S1 Pull-down assays with full-length gephyrin P2 and GlyR β-loop mutants

(**A**) Various P2 variants including WT P2 were purified and co-precipitated with WT GlyR β-loop beads. Bound protein in the pellet (upper panel) and unbound protein in the supernatant (lower panel) protein were subjected to SDS-PAGE analysis. The lane labeled M contains a molecular weight standard.

(**B**) Co-sedimentation of WT P2 with various GlyR β-loop variants immobilized to the beads.

Supplementary Table I. ITC parameters of Geph-E, P2 and GlyR β-loop mutants

Geph-E	N1	K_d1 (μM)	ΔH1 (kcal/mol)	N2	K_d2 (μM)	ΔH2 (kcal/mol)
WT	0.64	0.12	-16.1	0.65	7.8	-6.6
F330A	0.64	9.2	-10.3	n.a.[a]	n.a.	n.a.
PPAA	1.3	12.4	-12.7	n.a.	n.a.	n.a.
P713E	n.d.[b]	n.d.	n.d.	n.d.	n.d.	n.d.
P2 wt	0.35	0.09	-18.4	0.45	15.6	-8.3
P2 Y673F	0.31	7.4	-22.2	n.a.	n.a.	n.a.

GlyR β-loop	N1	K_d1 (μM)	ΔH1 (kcal/mol)	N2	K_d2 (μM)	ΔH2 (kcal/mol)
βWT	0.64	0.12	-16.1	0.65	7.8	-6.6
βF398A	0.82	14.2	-9.11	n.a.	n.a.	n.a.
βS399A	0.49	1.95	-18.3	n.a.	n.a.	n.a.
βI400A	0.42	2.63	-21.2	n.a.	n.a.	n.a.
βFI	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
βFIF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a]In case the binding isotherms could be fitted with a single binding site the values for the second site are non-applicable (n.a.), [b]whereas experiments where binding could not be detected have been labeled n.d. (not-detectable).

Supplementary materials and methods

Cloning, site directed mutagenesis, protein expression and purification

For recombinant expression in *E. coli,* the sequence encoding the E-domain of rat gephyrin (residues 318-736) was amplified by PCR from the gephyrin cDNA and cloned into the SapI/PstI restriction sites of the pTWIN1 vector (New England Biolabs). Point mutations were introduced into Geph-E (pTWIN1-$E_{318-736}$), full-length gephyrin (splice variant P2, pQE30-P2) and the GlyR β-loop (pTYB2-b49) using the QuikChange® site-directed mutagenesis kit (Strategene). For GFP-fused gephyrin variants, PCR amplified EcoRI/SalI fragments from pQE30-P2 plasmids with corresponding mutation were cloned into pEGFP-C2 vector. In a similar way, DsRed-tagged GlyR β-loop variants were PCR amplified from pTYB2-b49 variant constructs and inserted into BamHI/KpnI sites of pDsRed-N1 vector. Protein expression and purification of Geph-E and the GlyR β-loop followed the protocol described previously (Schrader et al., 2004). Selenomethionine (SeMet) substituted Geph-E was produced according to the feedback inhibition method (Doublié, 1997). Cells were grown at 37° C in M9 minimal medium composed of 48 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 0.4% glucose, 2 mM $MgSO_4$, and 0.1 mM $CaCl_2$. At an OD_{600} of 0.4, the amino acids lysine (0.1 mg/ml), phenylalanine (0.1 mg/ml), threonine (0.1 mg/ml), leucine (0.05 mg/ml), valine (0.05 mg/ml) and selenomethionine (0.05 mg/ml) were added. The culture was incubated at 37° C for 15 minutes, cooled down to 30° C, induced with 0.5 mM IPTG and incubated overnight. Purification of SeMet-substituted Geph-E and Geph-E variants was performed according to the same protocol utilized for non-substituted Geph-E (Schrader et al., 2004).

Supplementary references

Doublié S. (1997) Preparation of selenomethionyl proteins for phase determination. *Methods in enzymology* **276:** 523-530

Schrader N, Kim EY, Winking J, Paulukat J, Schindelin H and Schwarz G. (2004) Biochemical characterization of the high affinity binding between the glycine receptor and gephyrin. *J Biol Chem* **279:** 18733-18741