Supplementary Figure 1. pLGIC ECDs and AChBPs β-core-buried residue composition. Amino acids forming the β-cores in the crystal structures of nAChR-α1 (pdb entry 2QC1), GLIC (3EAM), *Lymnaea stagnalis* Ls-AChBP (1UV6) and *Aplysia californica* Ac-AChBP (2BYN) are plotted based on side-chain size and hydrophobicity. Only completely buried (ASA ≤ 5%) residues within 10Å (C_β-C_β distance) from either site 1 or site 2 residues are included. White bars are numbers of hydrophilic (threonine, serine, cysteine not engaged in disulphide bridges) and small hydrophobic (alanine) residues, black bars are bulky hydrophobic (valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan) residues.

Supplementary Figure 2. Comparison of β-core (ASA ≤ 25%) amino acid composition of pLGIC ECDs and AChBPs within 10Å (C_β-C_β) from either site 1 or site 2 residues. Data plotted for nAChR-α1, GLIC, Ls-AChBP and Ac-AChBP are based on crystal structures. For other eukaryotic pLGICS, putative β-core residues plotted are based on sequence alignments shown in Fig. 1. White bars are numbers of hydrophilic (threonine, serine, cysteine not engaged in disulphide bridges) and small hydrophobic (alanine) residues, black bars are bulky hydrophobic (valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan) residues.

Supplementary Table 1. List of amino acids in the β-cores of pLGIC ECDs and AChBPs within 10Å (C_β-C_β) from either site 1 or site 2 residues. Site 1 and site 2 amino acids are in red. Putative β-core residues of pLGICs for which crystal structures are not available are in italics. Residues with ASA ≤ 5% are in bold (the other residues have 5% ≤ ASA ≤ 25%).

Supplementary Table 2. Summary of FOLDX analysis. Completely core-buried (ASA ≤ 5%) residues in nAChR-α1, GLIC, Ls-AchBP and Ac-AChBP were mutated *in silico* to alanine, and $\Delta\Delta G_{folding}$ values were calculated using FOLDX. $\Delta\Delta G_{folding}$ values for site 1 and site 2 mutations are in red.

Supplementary Table 3. C_α-C_α and C_β-C_β distances of site 1 and site 2 residues in available crystal structures of nAChR-α1 ECD, GLIC, and AChBPs bound to agonists and antagonists as well as in apo form. Distances reported are the average from the individual distances in each of the five subunits forming the pentameric assembly (with the exception of nAChR-α1, which was crystallized in monomeric form).

Supplementary Table 4. Piperidine-4-sulphonic acid (P4S) EC_{50} values for WT and mutant $GABA_A$Rs. Values are mean ± SEM from n experiments. Ranges in I_{P4Smax} and $I_{GABAmax}$ currents elicited from oocytes expressing WT or mutant $GABA_A$Rs are indicated. Max current ratios from the same oocyte are mean ± SEM from n experiments. Values statistically different from WT are indicated (* $p < 0.01$; ** $p < 0.05$).

Supplementary Table 1.

	#1	#2	β-core buried side-chains within 10Å (C_β-C_β) from site 1 or site 2															
nAChR-α1	T52	S126	L35	L37	L40	V43	V50	V54	A122	F124	C128	C142	M144	—	—	Y203	F205	M207
$GABA_AR$-β2	L59	A134	I42	I44	I47	V50	Y57	M61	I130	T132	C136	C150	L152	—	—	L210	F212	L214
$GABA_AR$-α1	I61	A136	I44	V46	F49	V52	Y59	V63	L132	V134	C138	C152	L154	—	—	T214	F216	L218
$GABA_AR$-γ2	I74	A149	M57	V59	I62	V65	Y72	I76	L145	I147	C151	C165	L167	—	—	V225	F227	L229
$5HT_{3A}R$	T63	T149	V46	M48	I51	V54	L61	V65	L132	L134	C136	C152	L154	—	—	F215	V217	I219
GlyR-α1	V60	L136	I43	I45	F48	I51	Y58	I62	I132	L134	C138	C152	M154	—	—	A212	F214	L216
GLIC	V39	V110	I22	L24	C27	L30	F37	A41	F106	A108	—	L126	I128	—	F165	Y186	L188	—
Ls-AChBP	V50	F121	L33	F35	I38	V41	V48	F52	I117	Q119	C123	C136	I138	—	F171	V197	L199	F201
Ac-AChBP	L52	F125	F35	L37	I40	—	V50	Y54	Q121	L123	C127	C140	V142	F144	Y174	L200	V202	F204

Supplementary Table 2.

nAChR -α1	mutant	L35A	L37A	—	V50A	T52A	V54A	F124A	S126A	M144A	—	—	Y203A	F205A	M207A
	ΔΔG (kcal/mol)	4.01	3.56	—	2.22	0.68	2.42	4.38	–0.95	2.88	—	—	3.46	3.64	3.13
GLIC	mutant	I22A	L24A	C27A	F37A	V39A	—	F106A	V110A	L126A	I128A	F165A	Y186A	L188A	—
	ΔΔG (kcal/mol)	3.67	3.35	1.07	4.37	2.48	—	4.48	2.66	3.10	3.87	4.72	4.87	3.46	—
Ls-AChBP	mutant	L33A	F35A	I38A	V48A	V50A	F52A	I117A	F121A	I138A	—	F171A	V197A	L199A	F201A
	ΔΔG (kcal/mol)	3.52	4.63	4.44	2.60	1.94	4.14	3.01	4.48	3.77	—	4.08	3.05	4.31	5.56
Ac-AChBP	mutant	F35A	L37A	I40A	V50A	L52A	Y54A	L123A	F125A	V142A	F144A	—	L200A	L200A	V202A
	ΔΔG (kcal/mol)	5.16	4.21	4.04	2.49	3.84	3.78	3.30	4.29	2.50	4.69	—	3.76	3.76	2.62

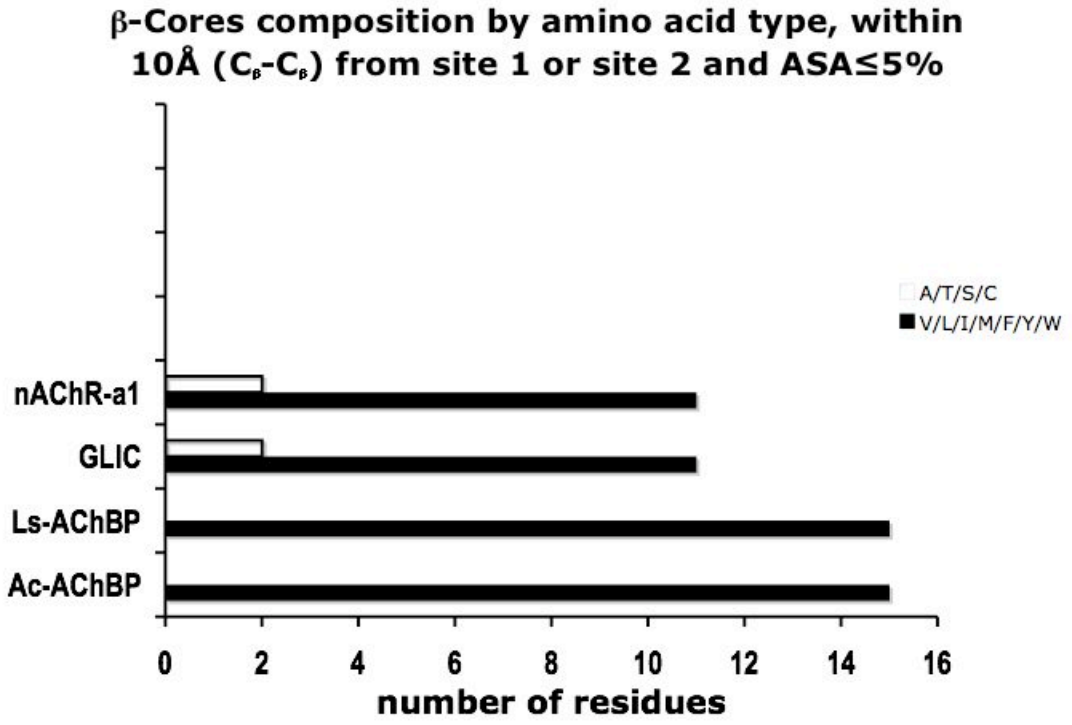
Supplementary Table 3.

	C_α- C_α	C_β- C_β	resolution
nAChR-α1 ECD	8.00Å	6.83 Å	1.94 Å
GLIC, pH4.6	7.44 ± 0.03Å	5.85 ± 0.02Å	2.90Å
GLIC, pH4.0	7.39 ± 0.02Å	6.11 ± 0.01Å	3.10Å
Ac_AChBP, apo form	8.28 ± 0.02Å	7.29 ± 0.04Å	2.02Å
Ac_AChBP, w/ EPI (+)	8.28 ± 0.03Å	7.32 ± 0.08A	3.40Å
Ac_AChBP, w/ LOB (+)	8.25 ± 0.08Å	7.21 ± 0.08Å	2.05Å
Ac_AChBP, w/ MLA (-)	8.27 ± 0.09Å	7.14 ± 0.29Å	2.45Å
Ac_AChBP, w/ lml (-)	8.29 ± 0.01Å	7.30 ± 0.04Å	2.07Å
Ac_AChBP, w/ PnIA (-)	8.36 ± 0.06Å	7.32 ± 0.08Å	2.40Å
Ls_AChBP, w/ HEPES	7.96 ± 0.03Å	6.58 ± 0.06Å	2.10A
Ls_AChBP, w/ CCh	7.97 ± 0.02Å	6.47 ± 0.03Å	2.50Å
Ls_AChBP, w/ nicotine	7.95 ± 0.02Å	6.66 ± 0.04Å	2.20Å
Ls_AChBP, w/ αCTX	7.86 ± 0.03Å	6.31 ± 0.05Å	4.20Å

Supplementary Table 4.

Receptor	P4S EC_{50}	n	I_{P4Smax} (μA)	$I_{GABAmax}$ (μA)	I_{P4Smax} / $I_{GABAmax}$	$I_{GABAmax}$ / I_{PBmax}	I_{P4Smax} / I_{PBmax}	n
WT	124.46 ± 7.38 μM	5	2.53 – 6.29	7.03 – 16.66	0.35 ± 0.03	1.75 ± 0.18	0.61 ± 0.11	7
αβA134Fγ	19.31 ± 3.36 mM	4	0.35 – 0.59	1.76 – 3.89	0.19 ± 0.03 **	0.63 ± 0.07	0.12 ± 0.03 *	8
αA136VβA134Vγ	475.30 ± 67.30 μM	3	0.44 – 4.33	2.80 – 14.58	0.24 ± 0.05	0.77 ± 0.02	0.18 ± 0.06 *	11

Supplementary Figure 1.



Supplementary Figure 2.