



Figure S1 **a**, nAChR is a hetero-pentameric receptor with two ligand binding sites (green) in its extracellular domain (ECD). Regions of ion selectivity are shown in red. A putative gate (purple) is located in the transmembrane domain. **b**, Homomeric AChBP is homologous to the ECD of the nAChR with five ligand binding sites. A, water filled *Ac*_AChBP vestibule¹⁵ is shown with one subunit removed. Vestibule waters are colored in yellow. Ten oxygen atoms at the constriction point are colored red and make a plane of two pentameric rings (108° angles) orthogonal to the five fold axis. **c**, Top view looking down the vestibule of AChBP.

Table S1 Data Collection & Refinement Statistics

<i>Data collection</i>	SO ₄ ²⁻ /cacodylate
Beamline	ALS/ 8.2.2
Wavelength (Å)	1.00
Space group	P4 ₁
a, b, c (Å)	98.4, 98.4, 265.7
Pentamer / asymmetric unit	2
Resolution range ¹	50 – 3.08 (3.19 – 3.08)
R _{merge} (%) ^{1,2}	13.1 (48.8)
Observations	294,191
Unique reflections	46,364
Completeness (%) ¹	100 (100)
Redundancy ¹	6.3 (6.3)
<I/σ> ¹	14.3 (4.0)
B-factor from Wilson plot (Å ²)	64.0
<i>Refinement</i>	
Resolution range (Å)	20 – 3.1
Protein atoms	17346
Solvent / PEG atoms	293 / 105
R _{cryst} (%) / R _{free} (%)	21.2 / 25.0
Free reflections	2,300
R.m.s. 1-2 bond distances (Å)	0.012
R.m.s. 1-3 bond angles (°)	1.441
Mean main / side chain B (Å ²)	50.1 / 50.8
Mean B solvent / PEG (Å ²)	17.5 / 69.9
PDB accession code	Xxxx

¹ Values in parentheses are for the highest resolution shell.

$$^2 R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_{hkl} - \langle I_{hkl} \rangle|}{\sum_{hkl} \sum_i \langle I_{hkl} \rangle}; R_{\text{cryst}} = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}$$