Supplementary Information for Vijayan, Sahai, Czajkowski and Biggin.

SI Table 1. List of interacting residues selected for QM calculations.

GluA2	GluK1	GluK2	GluN2A
Gly402	Glu426	Glu409	Glu394
Tyr405	Tyr429	Tyr412	Glu396
Tyr450	Tyr474	Tyr457	His466
Pro478	Pro501	Pro485	Ser492
Leu479	Leu502	Leu486	Leu493
Thr480	Thr503	Ala487	Thr494
Arg485	Arg508	Arg492	Arg499
Leu621	Leu641	Leu624	Arg618
Ile623	Ile643	Ile626	Thr631
Gly648	Gly668	Gly652	Gly664
Thr649	Ala669	Ala653	Thr665
Leu650	Val670	Val654	Val666
Asp651	Arg671	Glu655	Pro667
Ser652	Asp672	Asp656	Asn668
Gly653	Gly673	Gly657	Gly669
Ser654	Ser674	Ala658	Ser670
Thr655	Thr675	Thr659	Thr671
Lys656	Met676	Met660	Glu672
Thr667	Ser706	Asn690	Arg673
Phe668	Leu720	Phe704	Val694
Thr686	Leu721	Leu705	Ile709
Tyr702	Met722	Met706	Tyr710
Glu705	Glu723	Glu707	Asp712
Met708	Ser726	Thr710	Val715
Lys730	Lys747	Lys731	Tyr742
Tyr732	Tyr749	Tyr733	

SI Table 2. iGluR structures that were checked for the conserved water molecule in the W5 site.

Receptor	Subtype	PDB ID
AMPA	GluA2	1FTJ, 1FTK, 1FTL, 1FTM, 1FTO‡, 1FW0, 1GR2, 1LB8, 1LB9‡, 1LBB, 1LBC, 1M5B, 1M5C, 1M5D, 1M5E, 1M5F, 1MM6, 1MM7, 1MQD, 1MQG, 1MQH, 1MQI, 1MQJ, 1MS7, 1MXU, 1MXV, 1MXW, 1MXX, 1MXY, 1MXZ, 1MY0, 1MY1, 1MY2, 1MY3, 1MY4, 1N0T, 1NNK, 1NNP, 1P1N, 1P1O, 1P1Q, 1P1U, 1P1W, 1SYH, 1SYI, 1WVJ, 1XHY, 2AIX, 2AL4, 2AL5, 2ANJ, 2CMO‡, 2GFE, 2I3V, 2I3W, 2P2A, 2UXA, 3B6Q, 3B6T, 3B6W, 3B7D, 3BBR
Kainate	GluK1	1TXF, 1VSO, 1YCJ, 2F34, 2F35, 2F36, 2OJT‡, 2PBW, 3C31, 3C32, 3C33, 3C34, 3C35, 3C36
Kainate	GluK2	1S50, 1S7Y, 1S9T, 1SD3, 1TT1, 2I0B, 2I0C
NMDA	GluN2A	2A5S, 2A5T

‡ Structures that do not have a water at W5. These are low-resolution structures. The notable exception is 1FTO, the GluA2 ligand-free structure.

SI Table 3. Full breakdown of interaction energies (A) and basis set superposition errors (B) of water at site W5 in each QM system kcal mol^{-1}. Ws; all other waters (eg. for the W1-Ws cell, Ws means all other waters), L; ligand (glutamate), P; protein, LP; ligand and protein, WsL; waters and ligand, WsP; waters and protein, WsLP; waters and ligand and protein.

(A)

W5 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	0.84	-10.54	-6.10	-12.24	-5.84	-6.30	-9.53
GluK1	0.71	-11.38	-6.40	-14.28	-7.29	-6.39	-11.71
GluK2	0.88	-12.18	-7.26	-14.01	-8.45	-7.23	-12.22
GluN2A	1.86	-13.88	-18.66	-24.00	-10.06	-9.52	-30.80

(B)

W5 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	0.25	2.81	7.99	8.97	2.45	8.11	8.82
GluK1	0.24	2.74	8.72	9.58	2.39	8.83	9.44
GluK2	0.10	2.80	8.88	9.70	2.37	9.26	9.60
GluN2A	0.88	3.56	2.89	6.53	3.66	9.48	5.63

SI Table 4. Full breakdown of interaction energies (A) and basis set superposition errors (B) of water at the W6 site in each QM system kcal mol^{-1}. Ws; all other waters (eg. for the W1-Ws cell, Ws means all other waters, L; ligand (glutamate), P; protein, LP; ligand and protein, WsL; waters and ligand, WsP; waters and protein, WsLP; waters and ligand and protein.

(A)

W6 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	1.22	-2.86	-37.59	-37.39	-1.32	-35.16	-34.91
GluK1	0.94	-2.62	-37.00	-36.93	-1.20	-33.93	-35.12
GluK2	0.50	-1.55	-36.72	-35.64	-0.65	-32.52	-33.70
GluN2A	0.32	-3.70	-4.25	-19.83	-3.91	-6.82	-18.45

(B)

W6 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	0.00	0.48	10.89	10.59	0.41	10.62	10.48
GluK1	0.00	0.53	10.75	10.45	0.44	11.96	10.42
GluK2	0.00	0.51	10.28	9.19	0.45	11.28	10.47
GluN2A	0.00	2.44	7.25	8.60	2.37	10.84	10.45

SI Table 5. Full breakdown of dispersion interaction energies (A) and basis set superposition errors (B) of water at site W5 in the GluA2 and GluN2A QM systems (kcal mol^{-1}). Ws; all other waters (eg. for the W1-Ws cell, Ws means all other waters), L; ligand (glutamate), P; protein, LP; ligand and protein, WsL; waters and ligand, WsP; waters and protein.

(A)

W5 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	0.43	-10.27	-10.92	-17.01	-6.06	-11.48	-14.67
GluN2A	1.11	-13.40	-17.18	-28.04	-10.31	-15.75	-25.39

(B)

W5 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	0.26	2.70	7.89	8.78	2.39	8.00	8.62
GluN2A	0.91	3.40	6.99	8.83	3.58	7.75	8.83

SI Table 6. Full breakdown of dispersion interaction energies (A) and basis set superposition errors (B) of water at the W6 site in the GluA2 and GluN2A QM systems (kcal mol^{-1}). Ws; all other waters (eg. for the W1-Ws cell, Ws means all other waters, L; ligand (glutamate), P; protein, LP; ligand and protein, WsL; waters and ligand, WsP; waters and protein, WsLP; waters and ligand and protein.

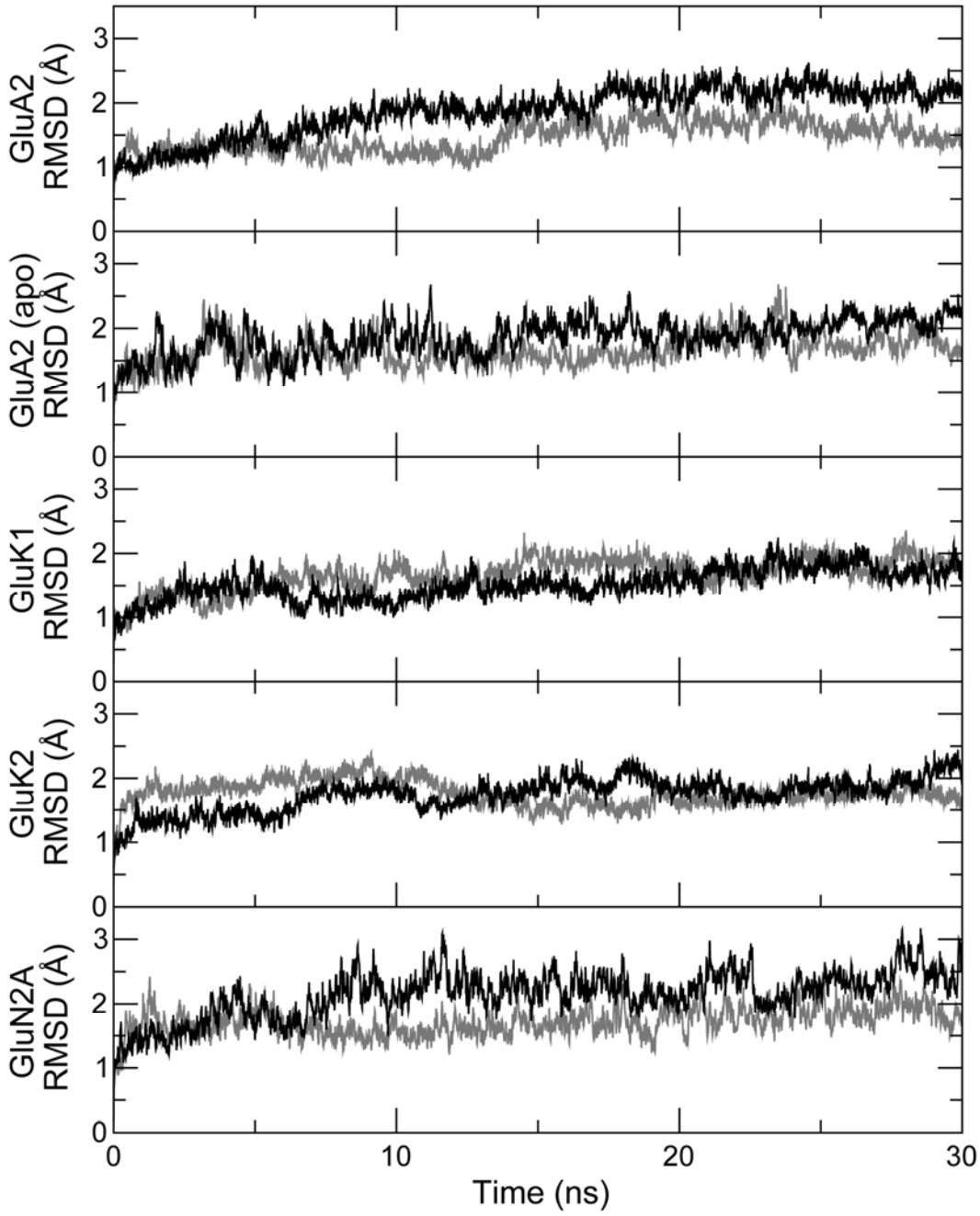
(A)

W6 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	1.09	-3.69	-40.90	-39.89	-2.24	-38.81	-37.88
GluN2A	0.27	-3.83	-11.05	-24.95	-4.03	-10.59	-24.48

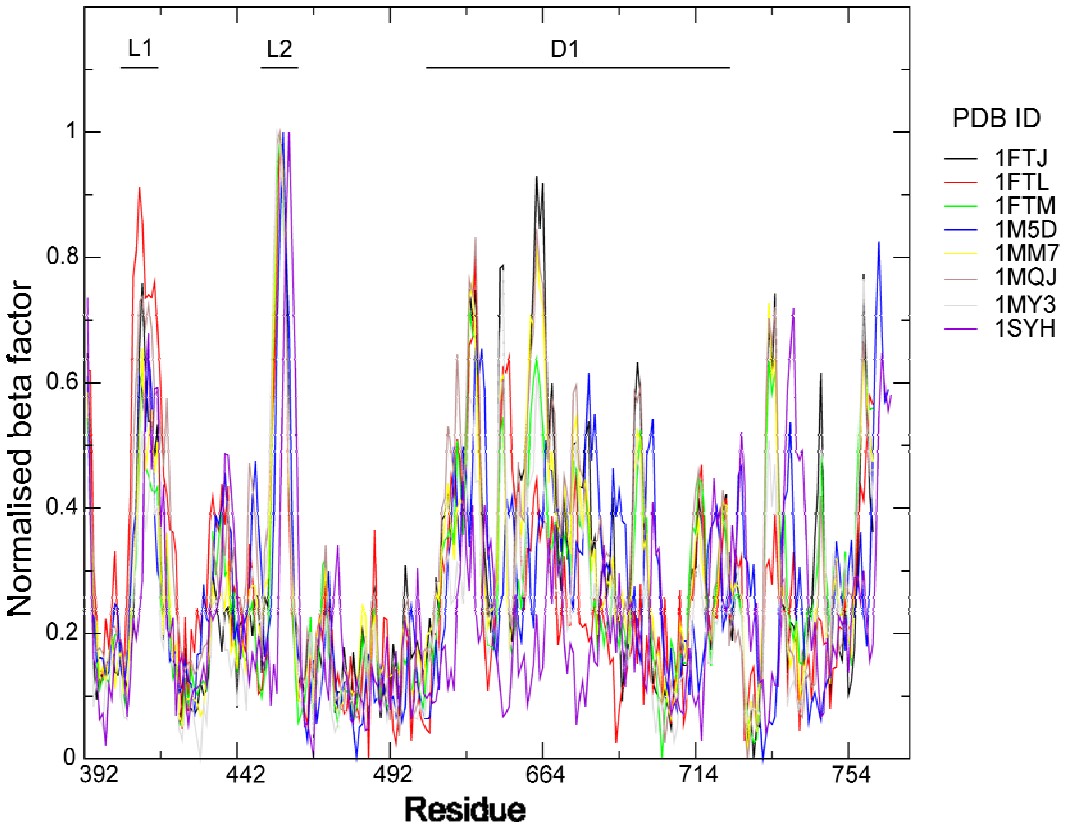
(B)

W6 Fragment	Ws	L	P	LP	WsL	WsP	WsLP
GluA2	0.00	0.51	10.64	10.35	0.43	10.39	10.23
GluN2A	0.00	2.73	6.82	8.76	2.45	6.79	8.59

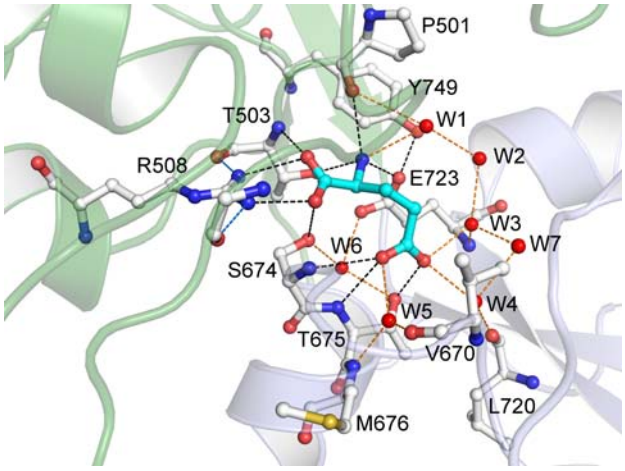
SI Figure 1. Root mean square deviation (RMSD) of all protein Cα atoms calculated as a function of time. All RMSDs plateau off indicating relaxation to a stable state.



SI Figure 2. Normalized beta factor from 8 GluA2 crystal structures. Regions of high disorder include loop 1 and loop 2 and most of domain D2.



SI Figure 3. Cartoon detailing the complex hydrogen-bond network between the ligand, protein and water (oxygen atoms only displayed) for GluK2.



SI Figure 4. The spatial density of water molecules calculated across the length of the second set of 20 ns MD simulations of (A) GluA2 (B) GluK1 (C) GluK2 and (D) GluN2A. Red spheres denote the location of crystal water molecules while the red mesh was computed from MD trajectories. The protein is shown in cartoon representation and the glutamate ligand in stick representation. All crystal water locations were preserved in MD simulations.