Defining the structural relationship between kainate receptor deactivation and desensitization

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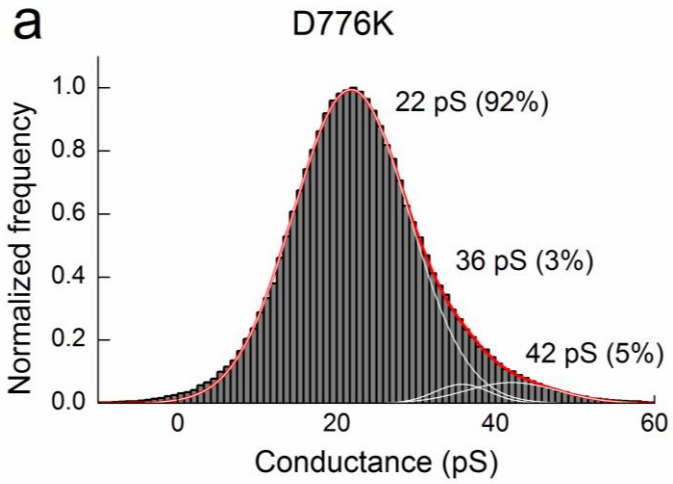
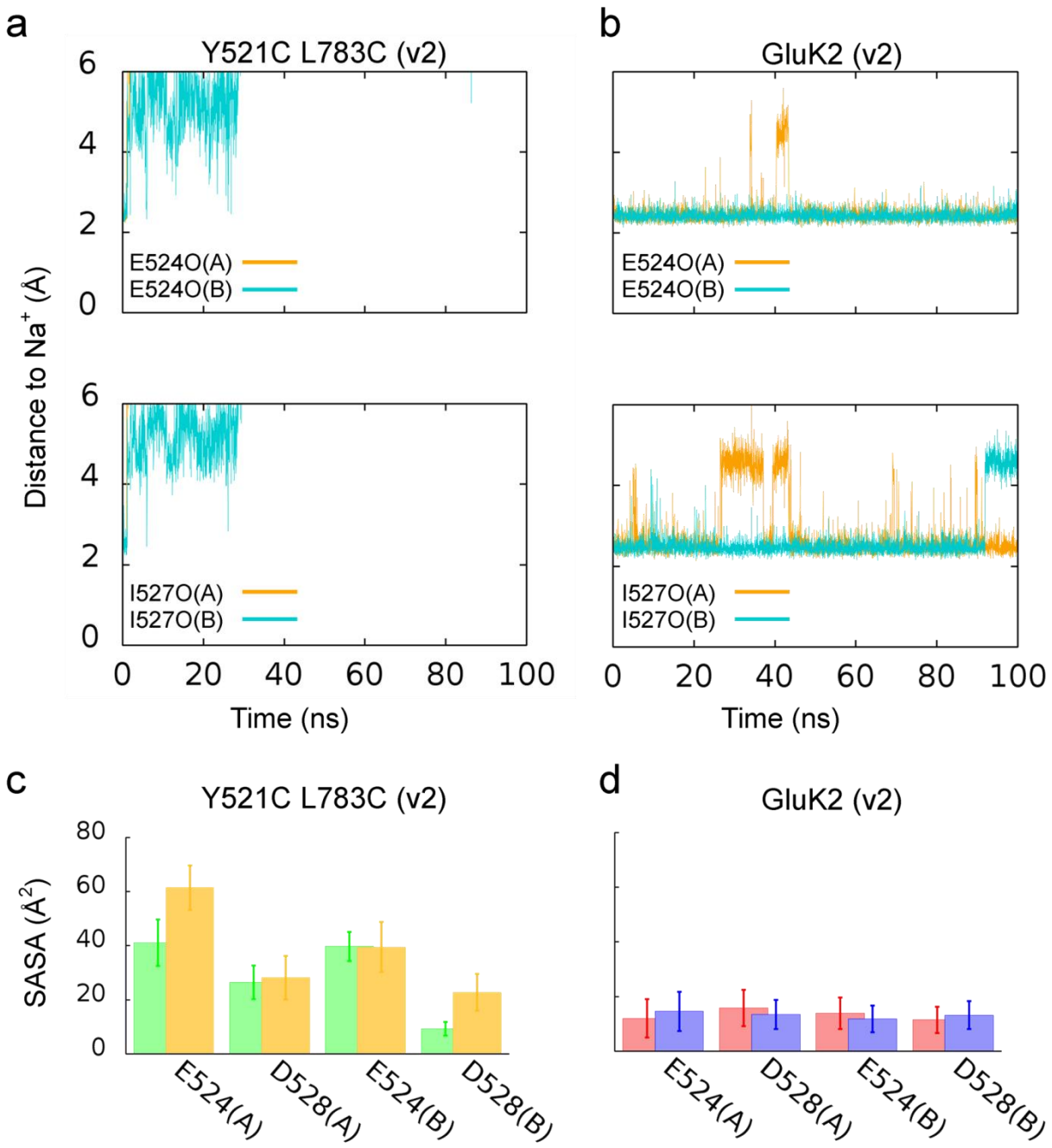
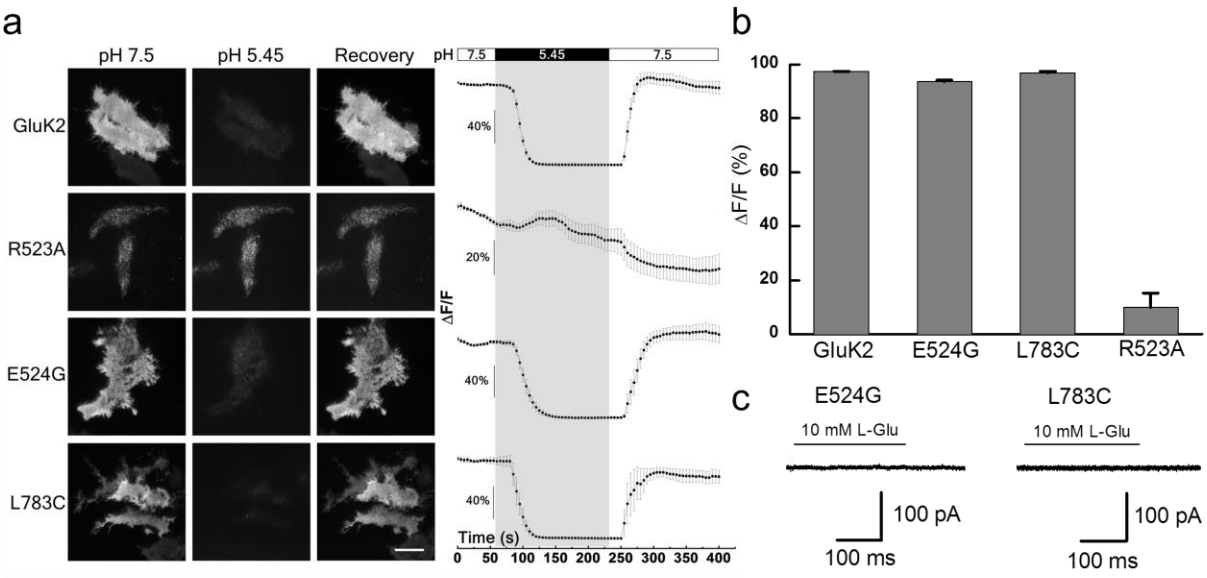
Supplementary figures



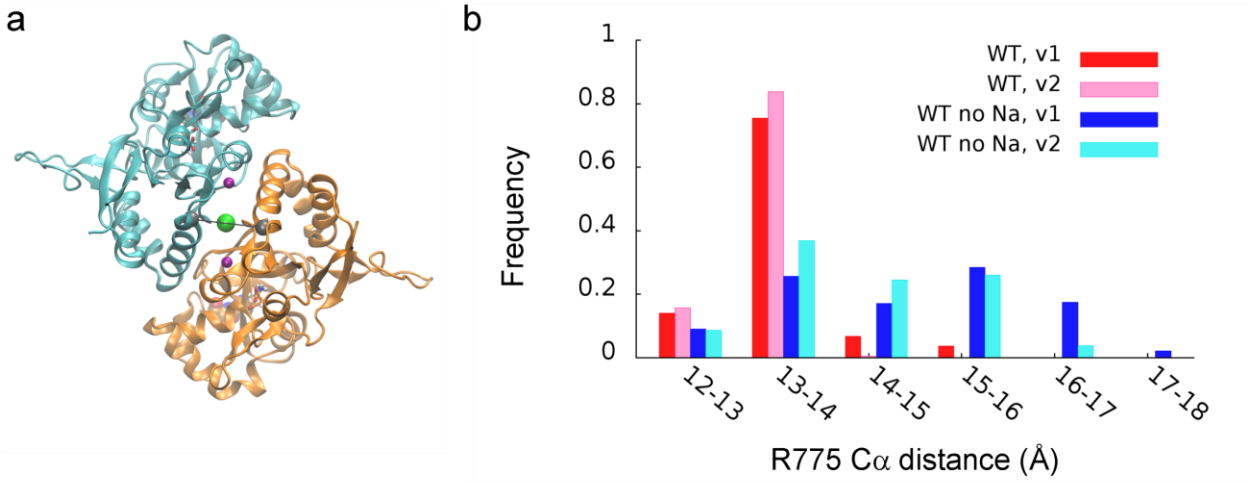
Supplementary Figure 1 The non-desensitizing receptor GluK2 D776K primarily activates to an approximately 20 pS conductance level. (**a**) An all-points histogram detailing the conductance distribution of D776K single channels. Conductance levels were identified by fitting the distribution with three Gaussian functions (see Methods). The distribution was generated by compiling segments of channel activity from the same patches and sweeps for which time course fitting was carried out, following digital low-pass filtering at 3 kHz.



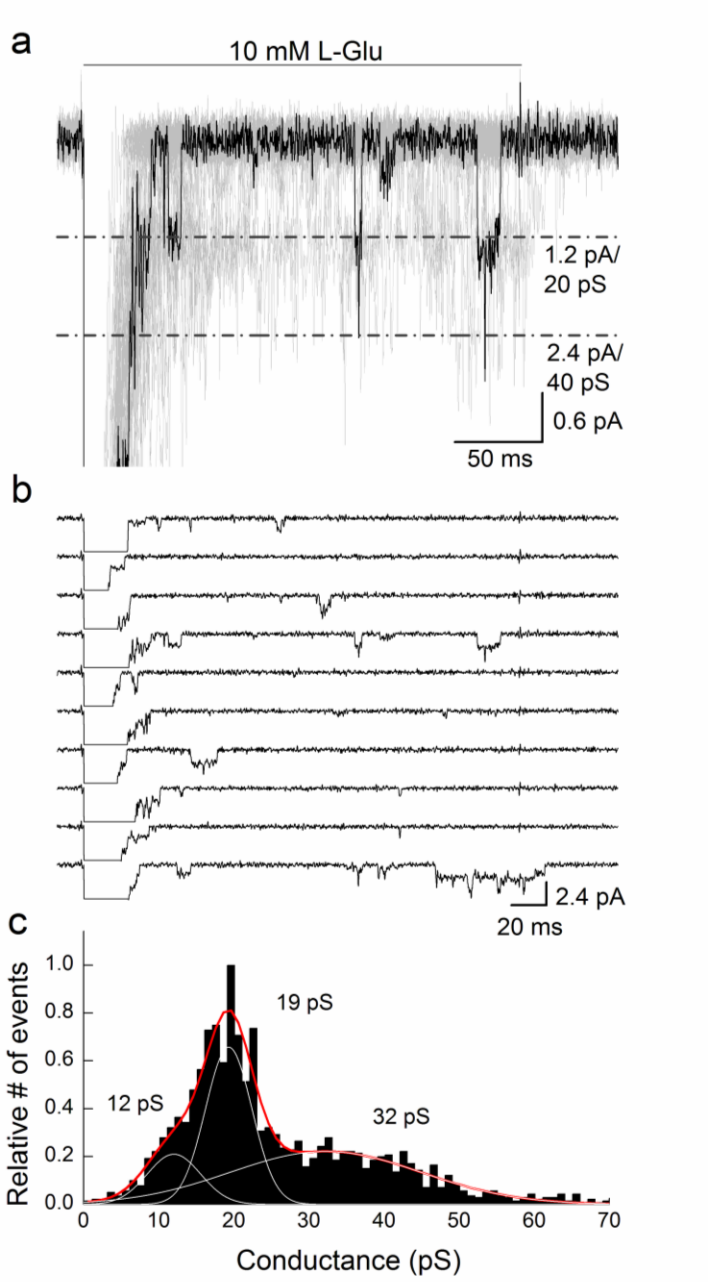
Supplementary Figure 2 Cation binding is predicted to be disrupted in the GluK2 Y521C L783C receptor. (**a,b**) Coordination of bound sodium ions by the backbone oxygen atoms of Glu 524 and Ile 527 in Y521C L783C (**a**) and wildtype GluK2 (**b**), respectively, as predicted by MD simulations. (**c,d**) Solvent-accessible surface area (SASA) for the sodium-coordinating residues Glu 524 and Asp 528 of Y521C L783C (**c**) and wildtype GluK2 (**d**). Mutant simulations are denoted by green (v1), orange (v2), and wildtype simulations by red (v1) and blue (v2) to distinguish different simulation versions.



Supplementary Figure 3 Mutations at the GluK2 cation binding pocket interfere with channel gating, but not receptor surface expression. (**a**) TIRF images of HEK293T cells transfected with wildtype and mutant GluK2 receptors reveal reversible changes in the eGFP fluorescence signal between pH 7.5 and 5.45 when proteins are expressed on the plasma membrane (scale bar = 10 μm). (**b**) Bar graph tabulating the changes in fluorescent signal observed when expressing wildtype and mutant GluK2 receptors. Error bars, s.e.m. from six to seventeen independent imaging experiments for each receptor. (**c**) Outside-out patch recordings taken from cells expressing GluK2 E524G (Patch # 12619p3) and L783C (Patch # 13114p5) receptors where 10 mM L-Glu failed to elicit a measurable current response.



Supplementary Figure 4 Sodium unbinding initiates rearrangements of the LBD dimer interface that are associated with desensitization. (**a**) Top view of the crystal structure of the wildtype GluK2 LBD dimer showing two sodium ions (purple), one chloride ion (green), and the alpha carbons of both R775 residues (grey) adjacent to the dimer interface. The distance of the line connecting these carbons reflects the separation of the upper lobes of subunits. (**b**) Inter-subunit R775 alpha carbon distance during 100 ns MD simulations in which the two bound sodium ions are either left in place or removed prior to the simulation.



Supplementary Figure 5 GluK2 channels activate to the same conductance levels before and after the onset of desensitization. (**a**) Overlay of forty individual current records from a patch expressing GluK2 receptors that produced a peak response of approximately 100 pA in 10 mM L-Glu (Patch # 12309p2, -60 mV). A typical opening elicited by L-Glu is shown in bold. (**b**) Typical GluK2 receptor unitary current events recorded from ten consecutive sweeps from the same patch, elicited by saturating L-Glu following the onset of desensitization. (**c**) GluK2 conductance distributions were fit following time-course fitting analysis of records from five patches (271 sweeps total).

Legends for supplementary videos

Supplementary Video 1 Lys 776 replaces sodium at the cation binding pocket of GluK2 D776K. MD simulations of the GluK2 D776K LBD dimer (with sodium initially bound) show that sodium (purple) is rapidly released from the cation binding pocket and eventually replaced by Lys 776. The chloride ion (green), however, remains bound. Additional details regarding simulation parameters are described in the online methods section and quantification of the MD results are shown in Figure 3. The video encompasses 100 ns of the simulation.

Supplementary Video 2 Allosteric ions are unstably bound at the GluK2 Y521C L783C LBD dimer interface. MD simulations of the GluK2 Y521C L783C LBD dimer show that sodium (purple) and chloride (green) ions are released from their respective binding pockets during the course of the simulation. Additional details regarding simulation parameters are described in the online methods section and quantification of the MD results are shown in Supplementary Figure 2. The video encompasses 100 ns of the simulation.

Supplementary Video 3 Mutation of the GluK2 cation binding pocket disrupts sodium binding. MD simulations of the GluK2 E524G LBD dimer show that both sodium ions (purple) are rapidly released from the cation binding pockets, while chloride (green) remains bound throughout the simulation period. Additional details regarding simulation parameters are described in the online methods section and quantification of the MD results are shown in Figure 5. The video encompasses 50 ns of the simulation.

Supplementary Video 4 Allosteric ions form stable interactions at the LBD dimer interface of wildtype GluK2. MD simulations of the GluK2 LBD dimer show that sodium (purple) and chloride (green) ions remain in their respective binding pockets during the course of the simulation. Additional details regarding simulation parameters are described in the online methods section and quantification of the MD results are shown in Figure 5. The video encompasses 100 ns of the simulation.

Supplementary Video 5 Mutation of the GluK2 LBD dimer interface disrupts sodium binding. MD simulations of the GluK2 L783C LBD dimer show water molecules (red and white) shielding sodium (purple) at the cation binding pocket. Eventually, one of two sodium ions is released from its binding pocket, while chloride (green) remains bound throughout the simulation period. Additional details regarding simulation parameters are described in the online methods section and quantification of the MD results are shown in Figure 5. The video encompasses 100 ns of the simulation.