Supplemental Data

Potent and Selective Inhibition of a Single AMPA Receptor Subunit by an RNA Aptamer

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Fig. S1

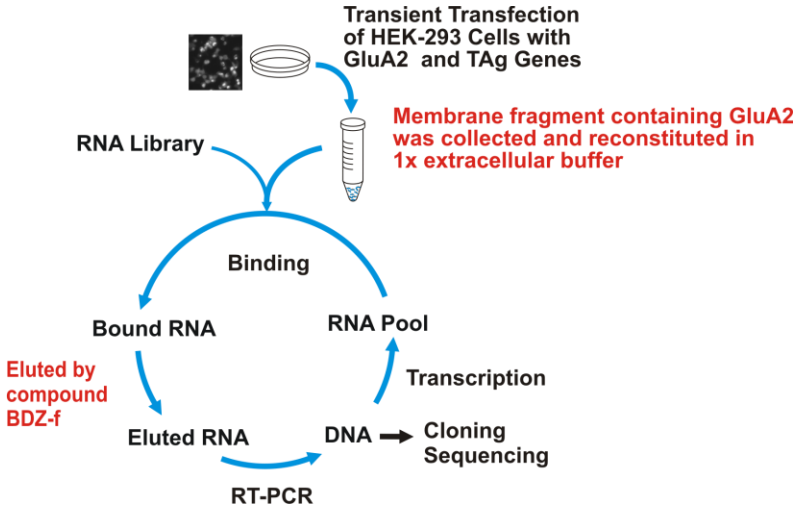

Figure S1. The use of SELEX, an in vitro evolution approach to identify aptamers selective to the closed-channel conformation of GluA2 AMPA receptors. The detailed method and operation have been described in the Experimental Procedure.

Fig. S2

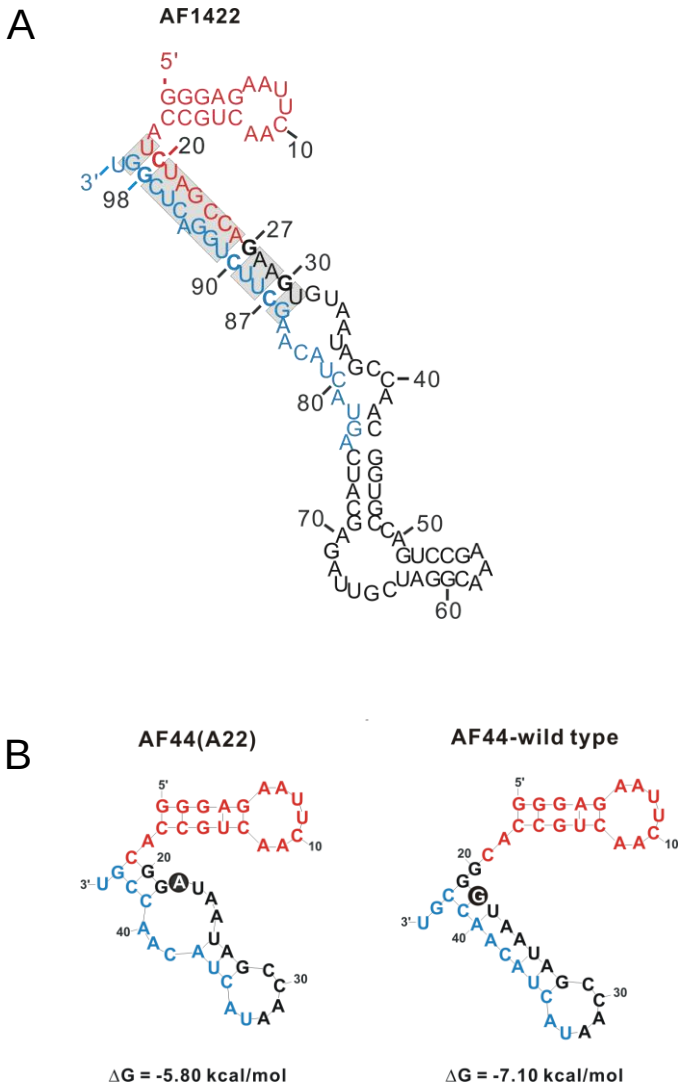

Figure S2. The secondary structures predicted by Mfold. (A) The shaded boxes indicate the nucleotides removed from the stem of AF1422 to form the short stem retaining only three-GC base pairs, i.e., C20/G98, G27/C90 and G30/C87 of AF4422. (B) The nucleotide G22 in the wild-type AF44 (right panel) was replaced with A22 (left panel) to maintain the two central loops as in AF1422. The resulting AF44(A22) (left panel) was termed AF44 for simplicity in this work.

Fig. S3

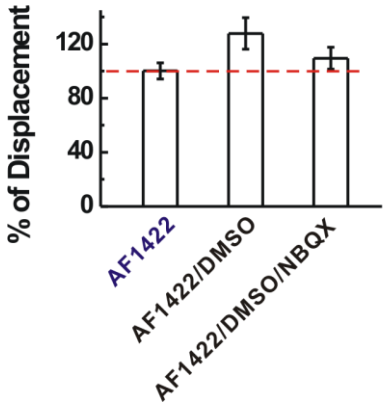

Figure S3. Percentage of displacement of the binding of AF1422 in the presence of DMSO and NBQX/DMSO. Binding of hot AF1422 without DMSO and NBQX was set to be 100 %. As a control, we also tested the effect of 8% DMSO on the binding of AF1422 to GluA2Q_{flip}; this was the amount of DMSO in the NBQX sample and DMSO was used to dissolve NBQX. Triplicate data sets for binding were collected and displaced as an avearge.

Fig. S4

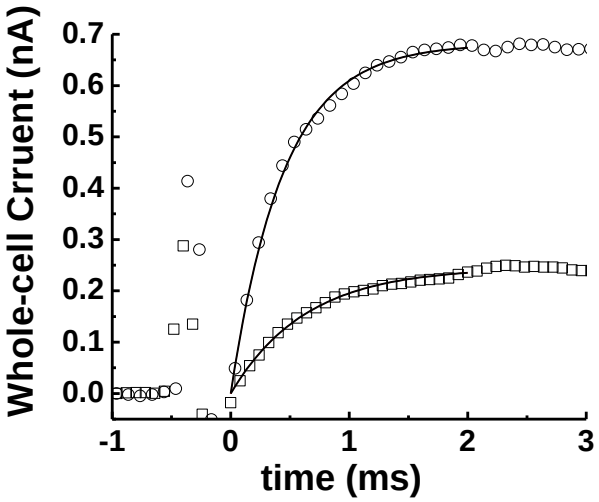

Figure S4. A laser-pulse photolysis measurement to show that only at a high concentration of AF44/AF42, the k_{obs}, which reflects k_{cl} or the open-channel conformation, can be inhibited. Specifically, at 100 μM photolytically released glutamate concentration, k_{obs} was determined to be 2204 ± 43 s^{-1} and the current amplitude through the $GluA2Q_{flip}$ channels was 0.68 nA in the absence of AF44/AF42 (upper trace). In the presence of 3 μM AF44/ AF42 (lower trace), k_{obs} was found to be 1490 ± 10 s^{-1} and the amplitude was 0.25 nA. This result shows that the open-channel conformation is inhibited by AF44/AF42, albeit weakly.

Fig. S5

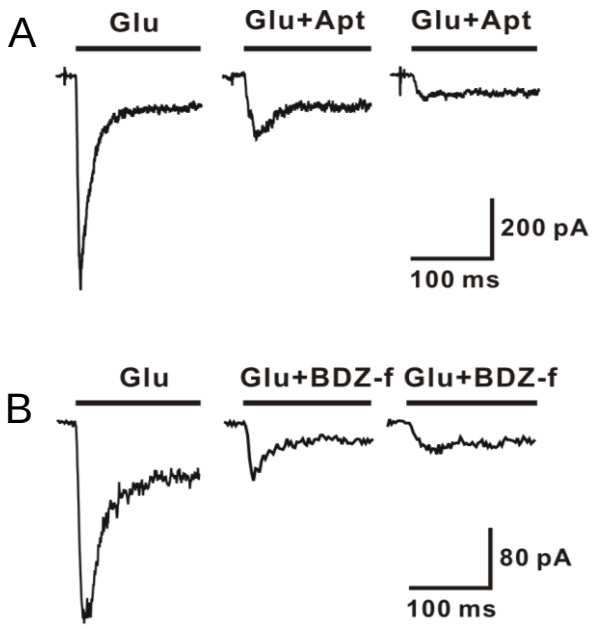

Figure S5. Inhibition of the whole-cell current response from the GluA2Q$_{flip}$ channels at very high concentrations of AF44/AF42 (A) and BDZ-f (B), where the non-desensitizing phase of the receptor response was uninhibited by either aptamer or BDZ-f, as shown by the trace in the right panel in both (A) and (B). All of the traces are drawn to the scale. The receptor response was evoked by 0.1 mM of glutamate in both (A) and (B). In (A), the AF44/AF42 used was 6.25 µM (middle panel) and 12.5 µM (right panel); in (B), the concentration of BDZ-f used was 10 µM (middle panel) and 20 µM (right panel).