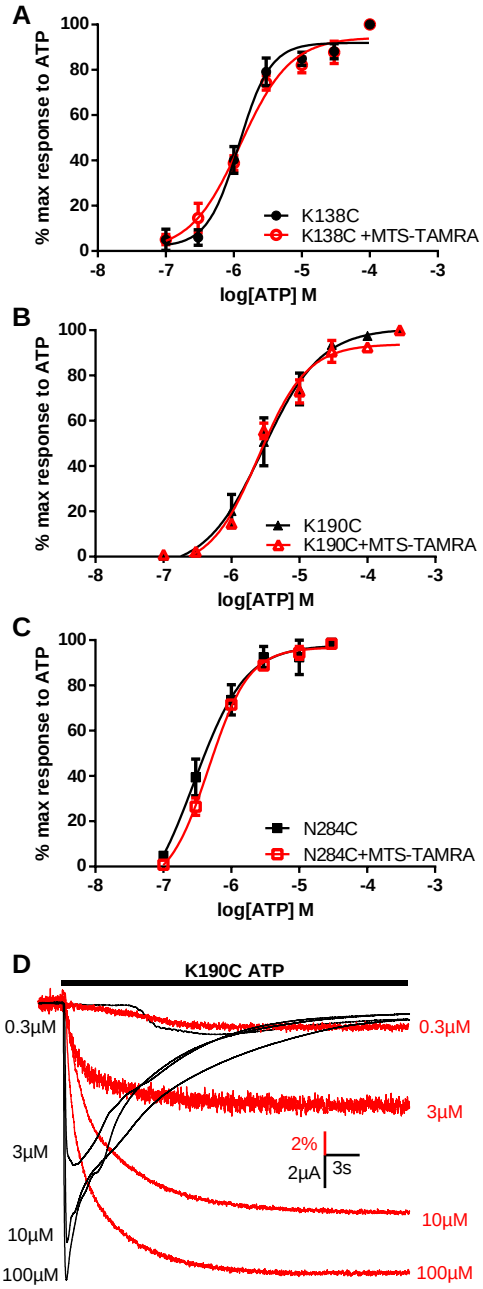


Kinetics of conformational changes revealed by voltage clamp fluorometry give insight to desensitization at ATP-gated human P2X1 receptors.

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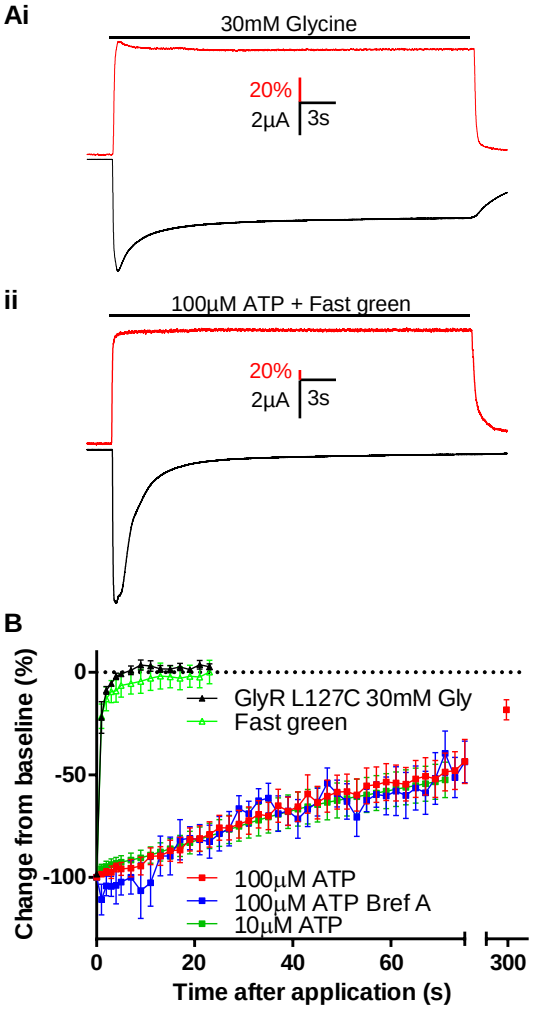
**Supplementary Figure 1. MTS-TAMRA labelling has no effect on agonist sensitivity at cysteine mutants.** Normalised ATP concentration response curves for current evoked at P2X1 receptor cysteine mutants K138C (A), K190C (B) and N284C (C) either with (red) or without (black) pre-incubation with MTS-TAMRA (n=4 for each). There was no change in sensitivity of the mutants to ATP when labelled with MTS-TAMRA. (D) Example VCF traces from oocytes expressing P2X1 receptor cysteine mutant K190C when challenged with various concentrations of ATP.

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**Supplementary Figure 2. MTS-TAMRA fluorescence recovery on agonist washout.** (A) Example VCF recording from oocytes expressing (i) GlyR L127C in response to glycine (30 mM, as indicated by bar) or (ii) P2X1 receptors in response to an ATP (100 µM) + fast green dye (150 µM) mixture. Fast green is fluorescence (and has no effect on ATP evoked responses) and can be used to estimate the time-course of solution exchange over the oocyte. In both cases there is a rapid increase in fluorescence, which is sustained during the application and returns to baseline levels quickly following washout. (B) The rate of fluorescence recover from ATP application at P2X1 K190C receptors was independent of ATP concentration (10 or 100 µM) and was also similar when oocytes have been incubated for 48 hours in Brefledin A to prevent receptor trafficking. GlyR L127C was used as a positive control that shows rapid MTS-TAMRA fluorescence recovery on glycine washout. An estimate of solution washout rate is shown for the dye Fast Green that had been applied to the oocytes.