The voltage-gated proton channel Hv1 has two pores each controlled by one voltage sensor

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Supplemental Information

Explanation for occurrence of events with reduced numbers of bleaching steps

In our subunit counting experiments with dimeric proteins (NMDAR with NR1+NR2-GFP) we observed that 1/3 of the fluorescent spots had only 1 bleaching step, even though 2 bleaching steps were expected. This reduced number of bleaching steps can be attributed to a ~20% probability that the GFP tag is not fluorescent. In our previous studies, we also observed this fraction of non-fluorescent GFP when tetrameric CNG-channels or both subunits in NMDA receptors were tagged. Although we observed slightly lower fractions of non-fluorescent GFP (around 10%) in some single experiments, the average value determined from 9 experiments with NR1 and NR2-GFP in this paper (22%) and the values from tetrameric channels in our previous publication (20%) reflect more accurate values.

In the case of 80% fluorescent GFP, the expected fraction of events with 1 bleaching step can be calculated as follows:

2-step events: 80% * 80% = 64%

1-step events: 2 * 80% * (100% - 80%) = 32%

0-step events: (100% -80%) * (100% - 80%) = 4%

1-step events / all visible events = 32% / (32% + 64%) = 1/3

We do not know the photophysical or molecular mechanism for non-fluorescence of some of the GFPs. It could be due to incomplete chromophore maturation, residence of the chromophore in a dark state, premature termination of transcription or translation, or posttranslational modification like cleavage, or a combination of all of these.

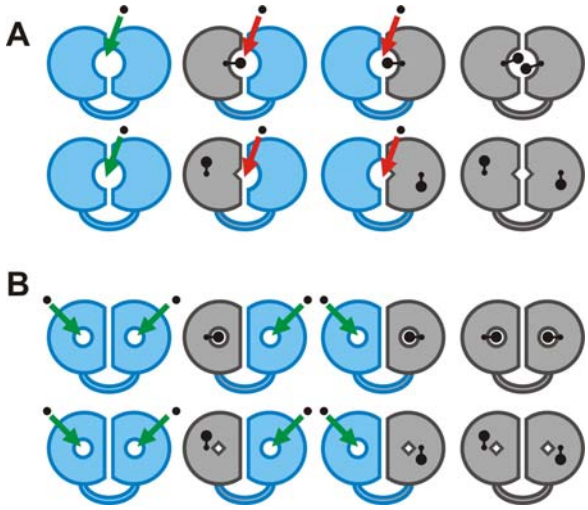
Effect of MTS modification on the G-Vs of channels containing the N214C subunit

We tested the effect of MTS modification on the voltage sensitivity of channels containing the N214C subunit comparing G-Vs before and after MTS treatment. Figure 4 shows that MTSET modification of the heterodimer WT-214C and homodimer 214C-214C reduces the maximal

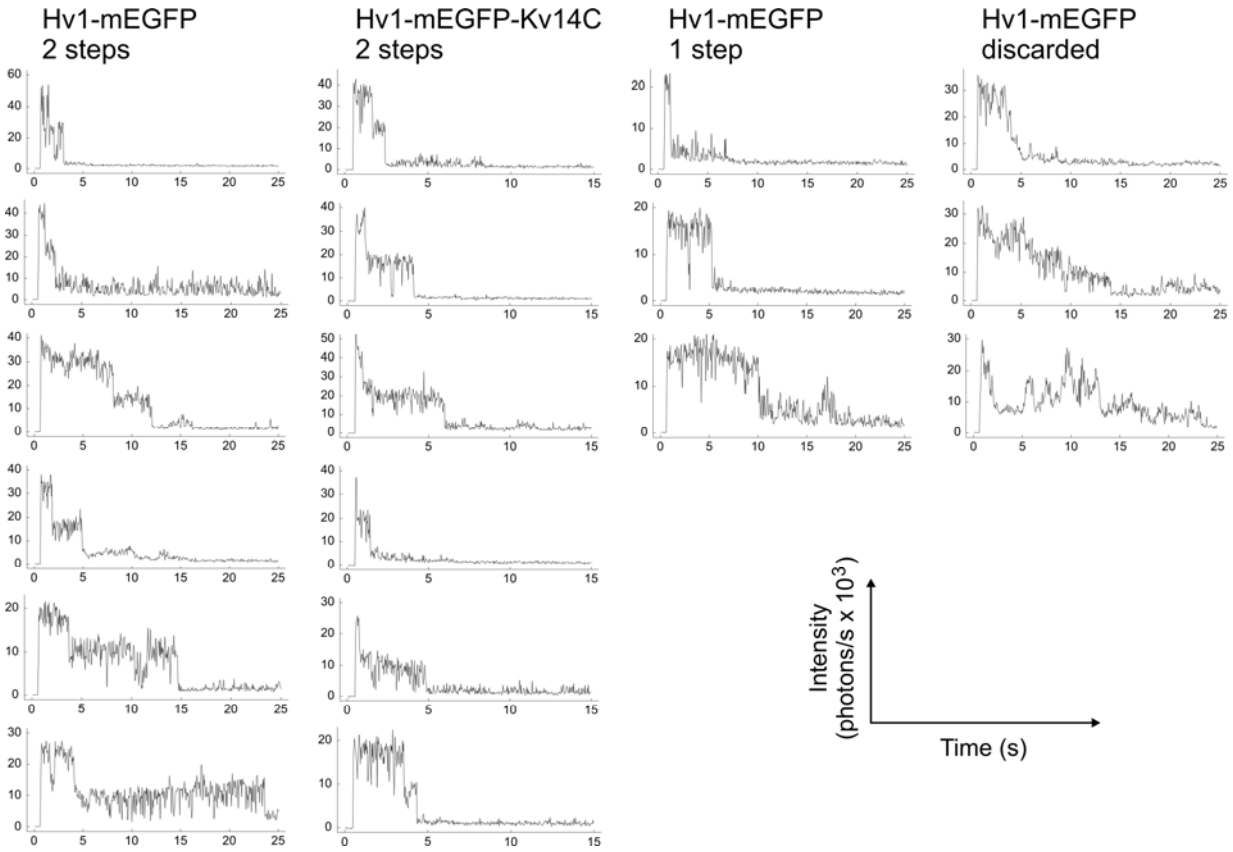
conductance by ~40% and ~95%, respectively (symmetrical pH 6.0), with minor shifts of the G-Vs. Once the G-Vs that were determined after MTSET treatment are scaled to the size of the initial G-Vs (no-MTSET), they are seen to closely overlay (Fig. 4A, B, grey and black symbols). Similar results were obtained with GEGETS and MTSACE (not shown). The test steps to +120mV or +140mV (blue bars in Figure 4A and B), which were used to monitor the effect of the MTS reagents, were chosen for being at the top of the G-Vs, where both unmodified and modified channels are maximally open. The residual tail currents of the homodimer 214C-214C after MTSET treatment decay much slower than the control (no-MTSET) currents (Figure 4C), allowing us to determine the degree of completeness of MTS modification. We found that the residual tails are uniformly slow, indicating complete modification by MTSET.

Direct versus allosteric effect of MTS modification of position N214C

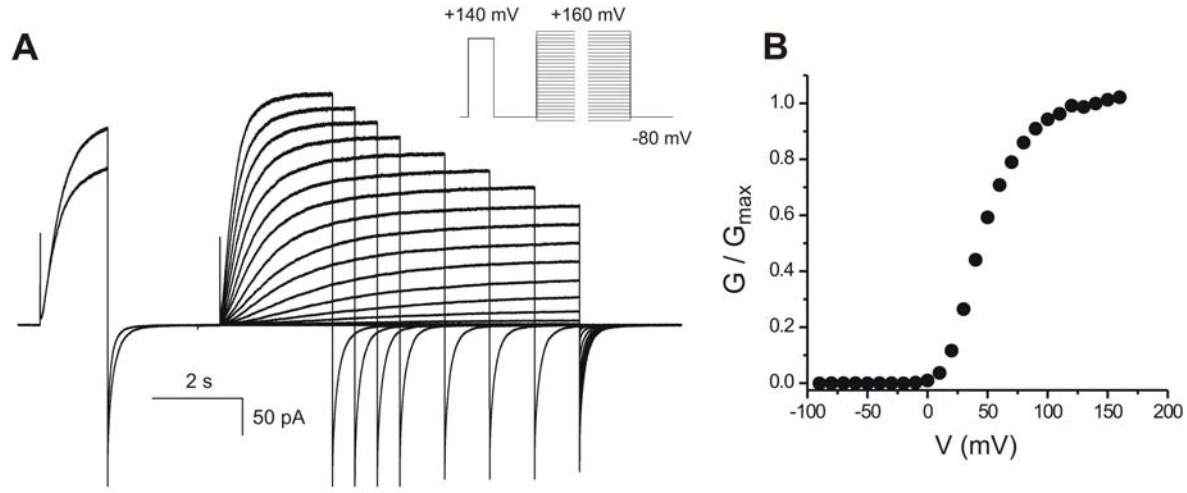
The analysis of the block by MTS reagents and guanidinium of heterodimers WT-214C and 214C-WT and homodimers WT-WT and 214C-214C argues that there are two pores per dimer. In Figure 3A we have depicted position N214C as located in the proton permeation pathway because our data strongly suggest so (see main text). However, it is important to note that the conclusion that there are two pores per dimer does not depend on where exactly N214C is located. As shown in Supplemental Figure 1, our data are inconsistent with the presence of one common pore per dimer also in the event of N214C being located outside the pore and the MTS modification acting allosterically on the pore.



Supplemental Figure 1 Expected inhibitions of MTS-modified channels by guanidinium in homo- and heterodimeric channels that have one pore (A) or two pores (B). WT subunit shown in blue, N214C subunit shown in gray. Tandem dimers with defined stoichiometry shown after MTS modification of 214C (black dots). Green arrows indicate WT guanidinium block. Red arrows indicate modified/perturbed guanidinium block. The upper row in each panel represents the case of direct block of the pore by the MTS reagent. The lower row represents indirect block due to MTS-induced structural changes in the pore.



Supplemental Figure 2. Examples of photobleaching traces from fluorescent spots for different Hv1 constructs. Shown are events with 2 bleaching steps, 1 bleaching step, and events that were discarded due to irregular emission, where no steps could be counted.



Supplemental Figure 3. G-V measurements from tail currents and rundown correction. A) Representative current traces from WT-214C tandem dimer showing the voltage protocol used to determine G-Vs (pH_i=pH_o=6.0). Traces are shown without leak subtraction and after low-pass digital filtering at 0.5 kHz. Tails after depolarization in a control pre-step to +140mV were used to correct for current rundown. For clarity, only the first and last traces elicited by the pre-step are shown. In this example, the final control tail current was decreased by 33% compared to the initial current (average rundown: 5.8 pA/min). The G-V resulting from the measurement shown in (A) had a $V_{1/2}$ = 45.5mV and a (kT/ze_o) = 16.0 mV. The protocol was repeated on the same patch and another G-V calculated ($V_{1/2}$ = 45.5mV and a (kT/ze_o) = 15.2 mV). The average G-V from these two measurements is shown in (B).