# Supporting Information for: Markov modeling reveals novel intracellular modulation of the human TREK-2 selectivity filter

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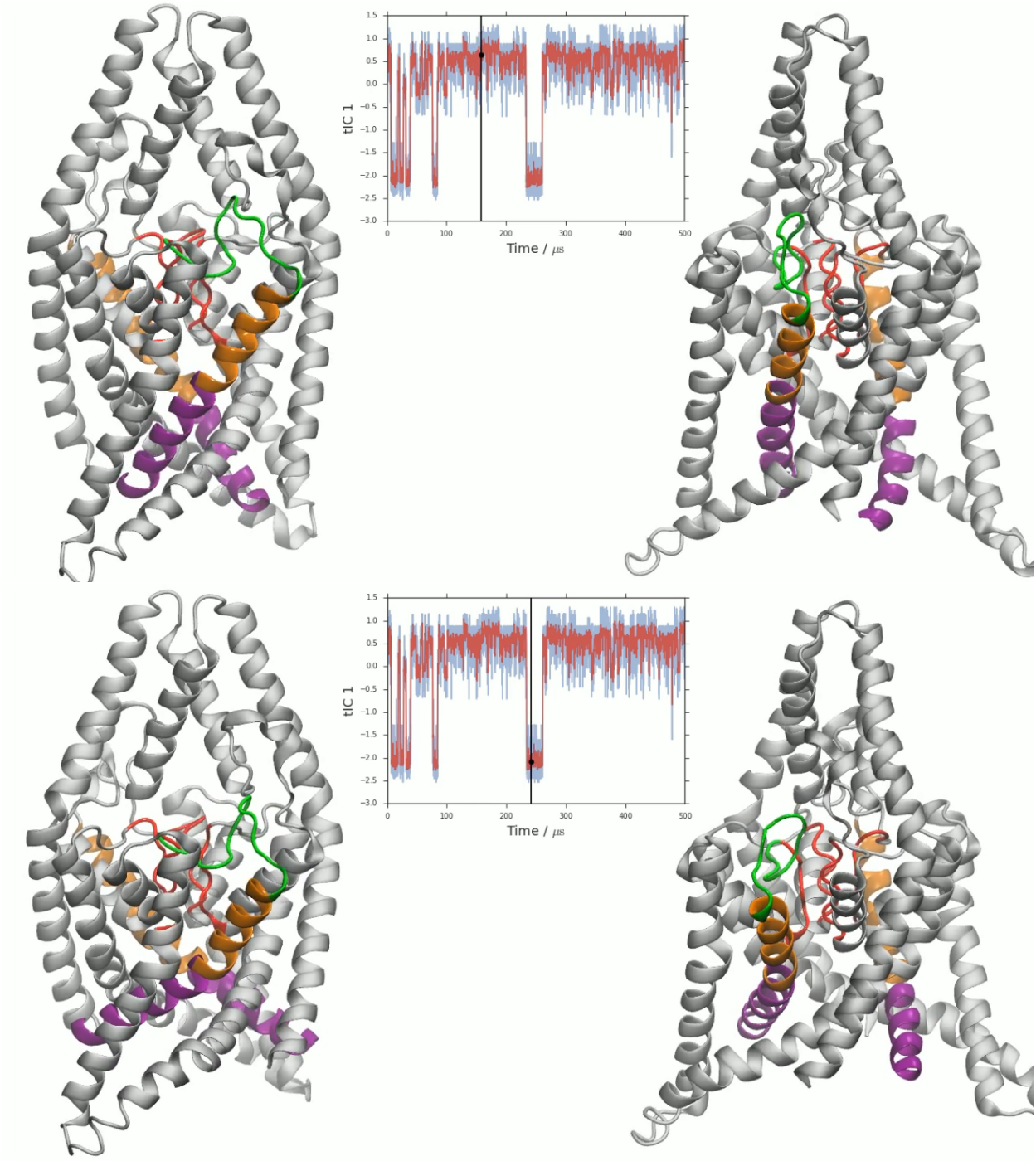


FIG. S1: We generated a 500 µs representative MSM trajectory by sampling among discrete states according to the modeled transition probabilities. The movie can be viewed in the supplementary file `trek.mp4`. Depicted here are two frames from the movie (top and bottom). Each frame consists of a view of the protein and its 90 degree rotation. We plot the projection of the conformation against its tIC 1 (Up-Down) coordinate. The black vertical line traces the current time in the trajectory.

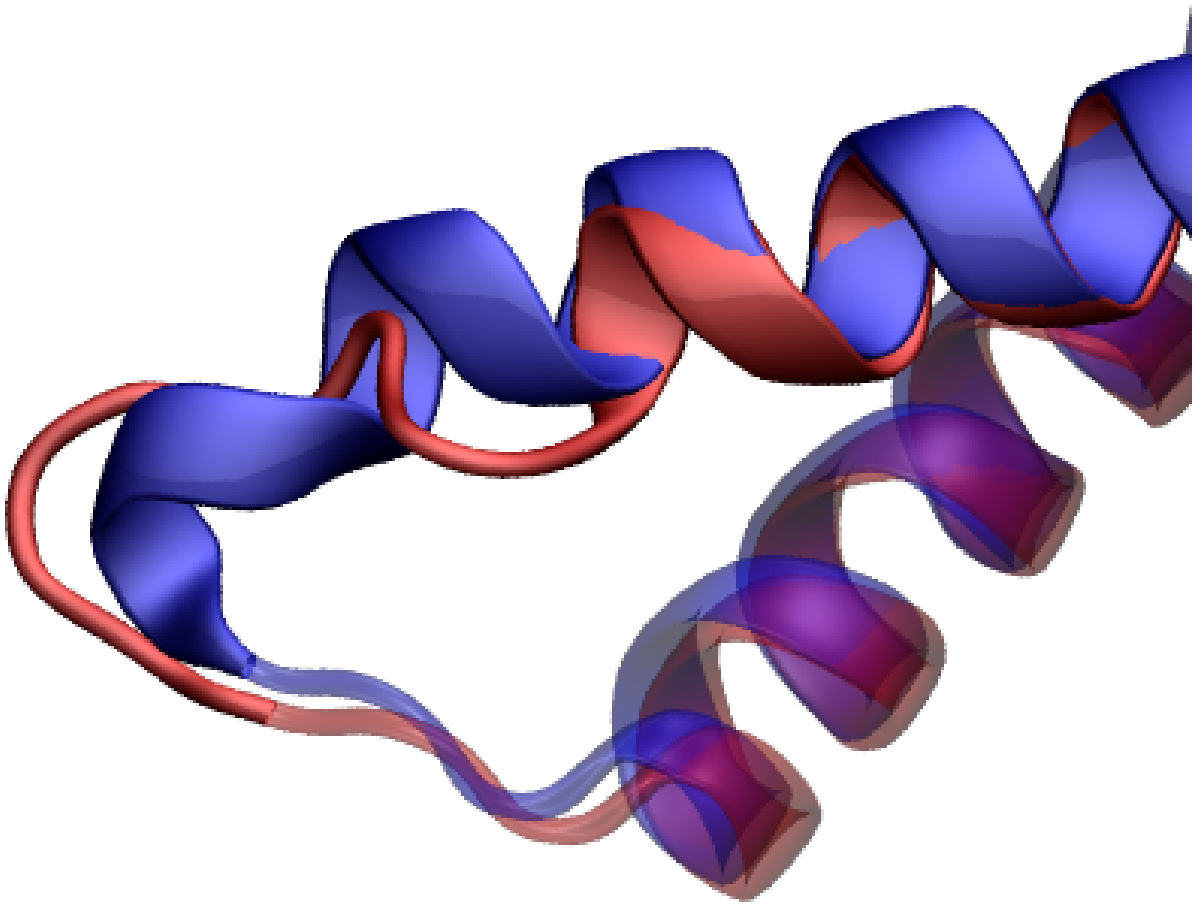

FIG. S2: Partial unfolding of the M2-M3 loop distinguishes $I_2$ from *Down*. Colors are as in Fig. 1B.

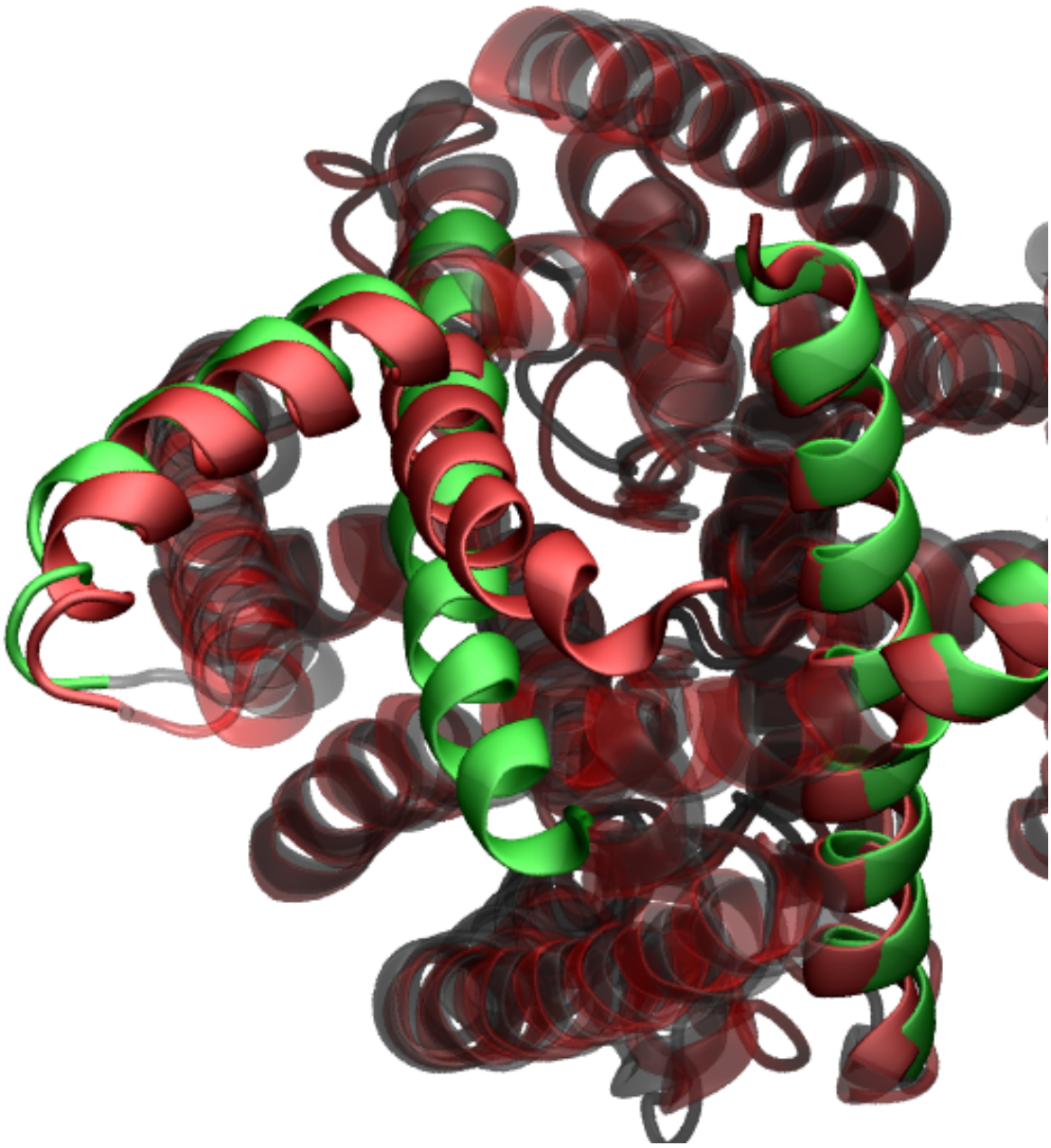

FIG. S3: $I_1$ and $I_2$ differ in positioning of the M4 helix. $I_2$ adopts the down-like configuration of this helix whereas $I_1$ adopts an an up-like configuration of this helix. Both intermediates adopt the down-like M2-M3 helix positions. From a purely structural standpoint, $I_1$ may be suggested to be "half down or half up". However, it is strongly kinetically related to the down conformations. Specifically, we observe a rapid relaxation from $I_1$ to $I_2$. Colors are as in Fig. 1B.

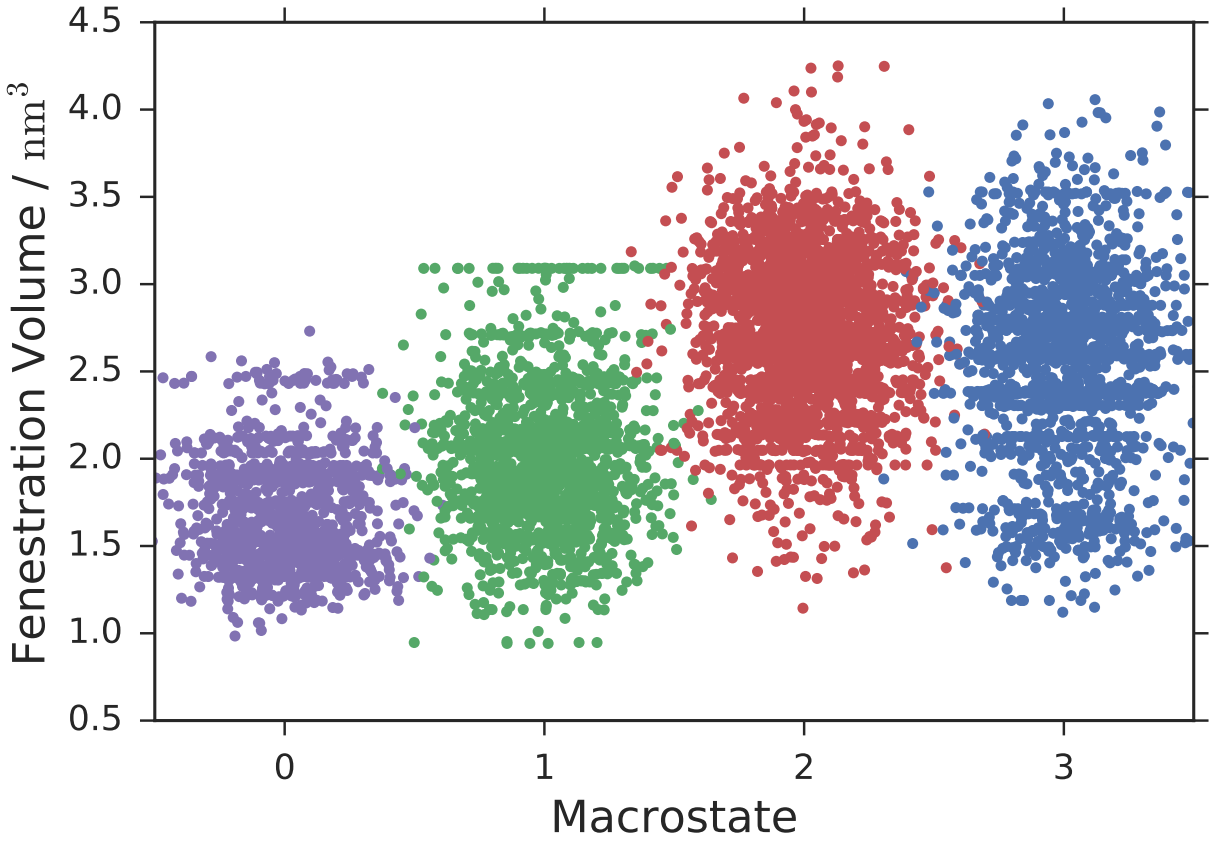


FIG. S4: Fenestration volume increases in macrostates $I_2$ and *Down*. Large fenestrations are created in the down conformations. Colors are as in Fig. 1B.

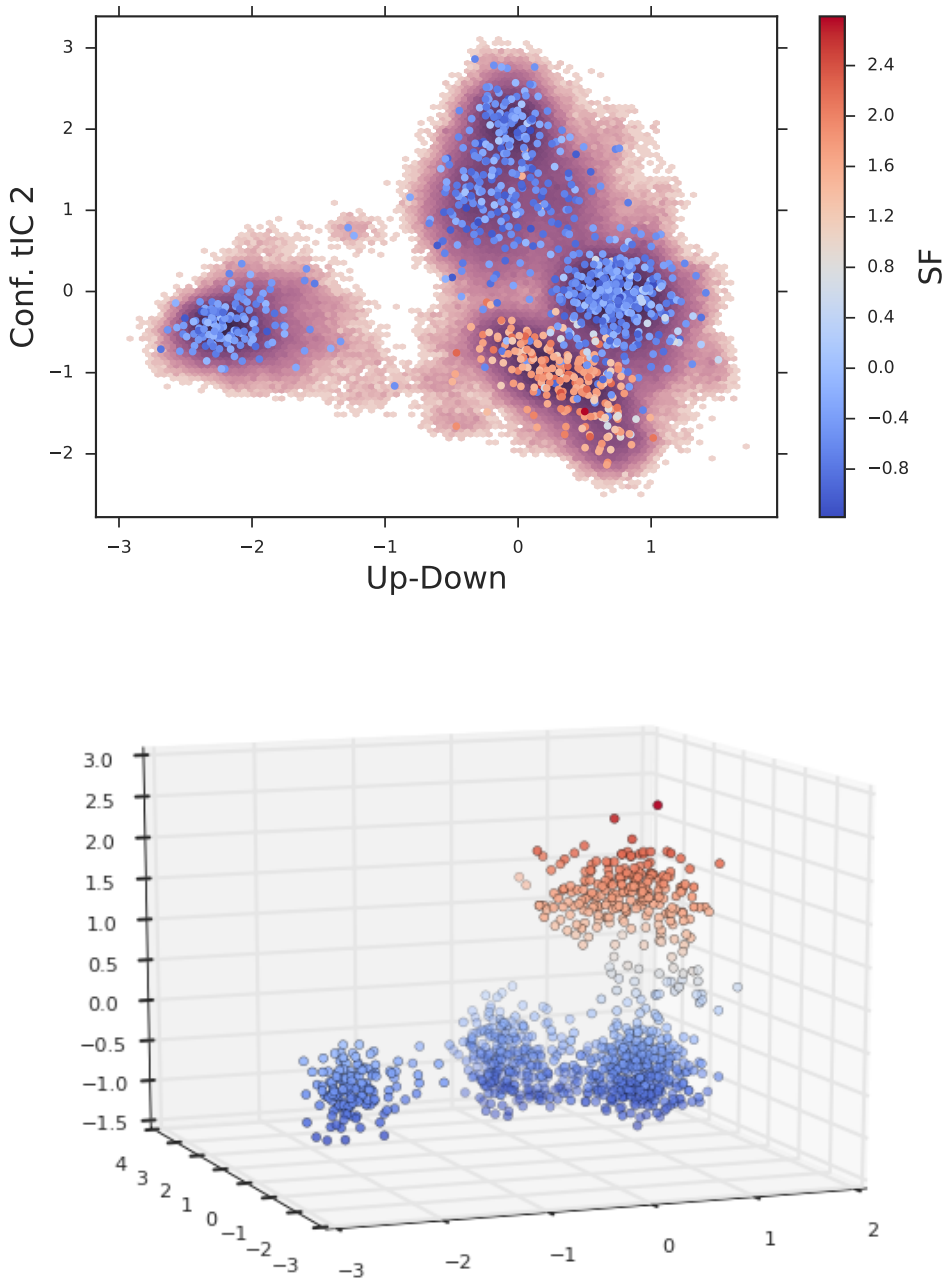


FIG. S5: We attempt to plot the three-dimensional histogram of observations versus the two conformational tICs and the SF tIC. (top) SF tIC value of cluster centers given by color. (bottom) 3D scatter plot of cluster centers, again colored by SF tIC value. These plots can be seen as a combination of Fig. 1B and C. The coupling is shown by the inset table in Fig. 1C.

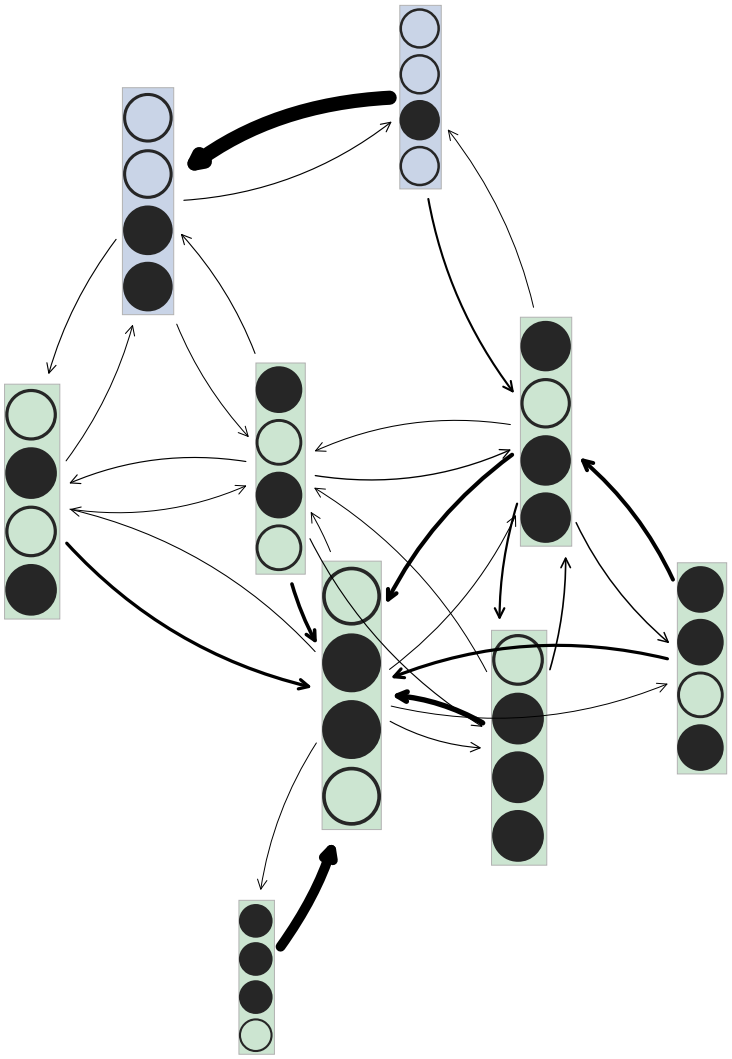

FIG. S6: Compare with Fig. 2 A and C. Whereas those figures show ion microstate transition graphs partitioned by *Up* and *Down* macrostates (resp), here we plot the microstate transition graph for the whole dataset without partitioning by macrostate.

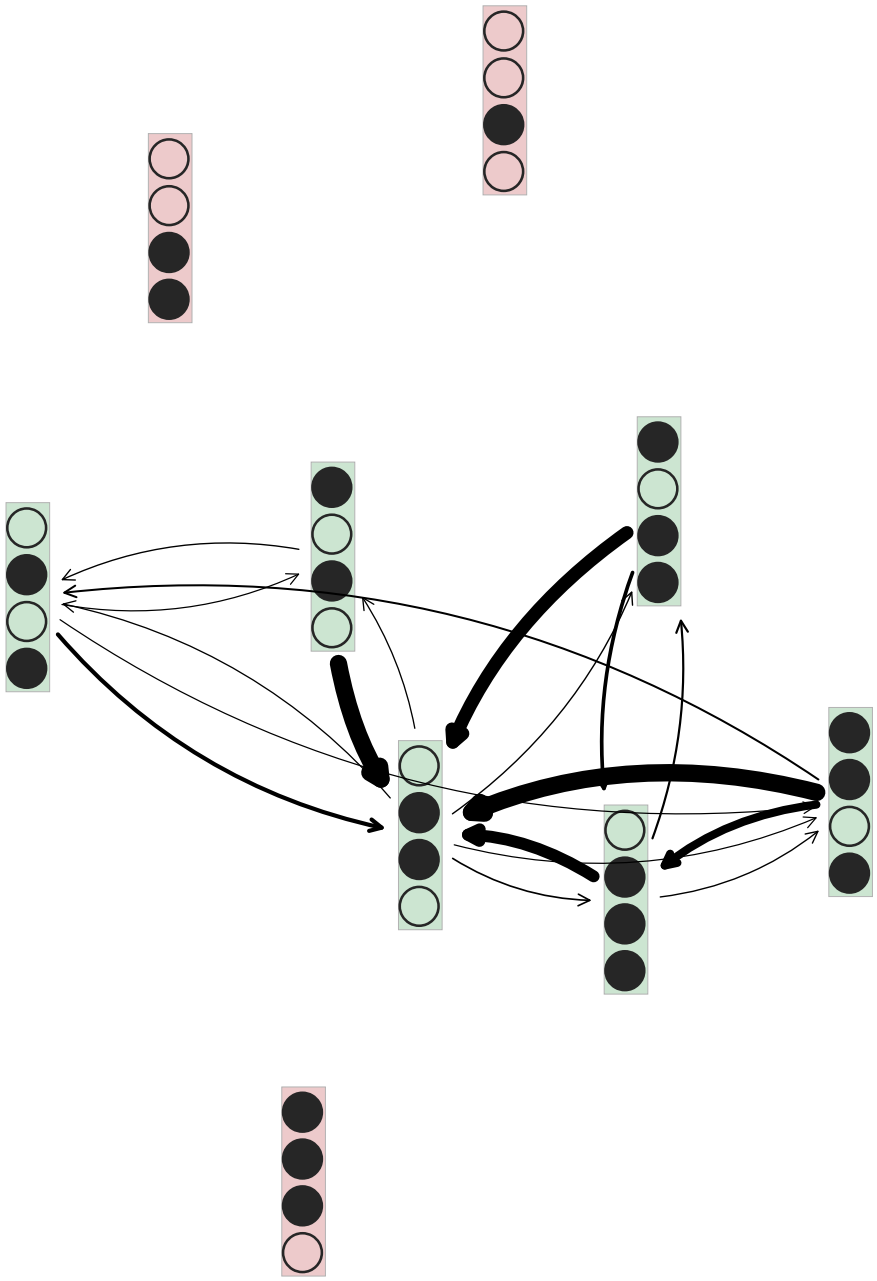

FIG. S7: Compare with Fig. 2 A and C. Whereas those figures show ion microstate transition graphs partitioned by *Up* and *Down* macrostates (resp), here we plot the microstate transition graph for the $I_1$ macrostate.

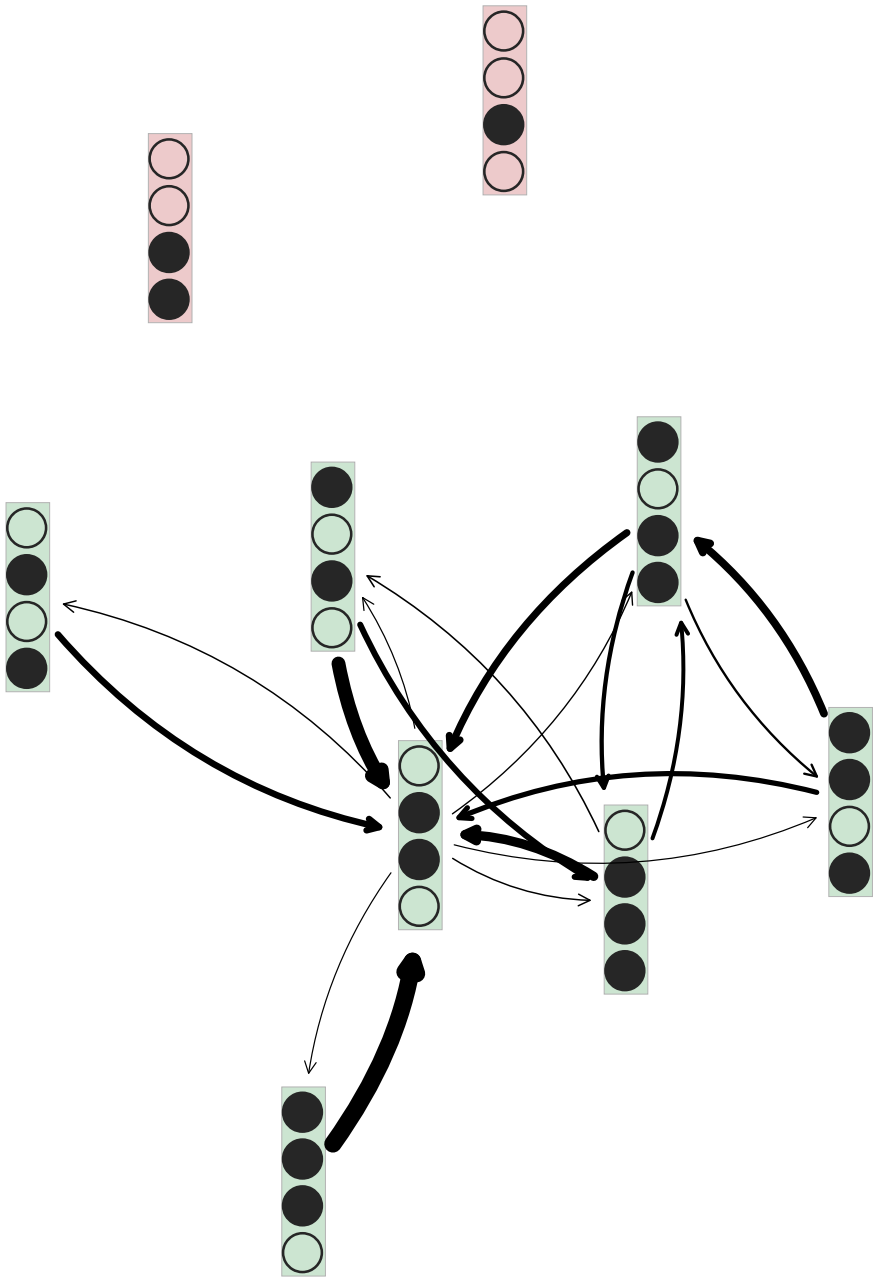

FIG. S8: Compare with Fig. 2 A and C. Whereas those figures show ion microstate transition graphs partitioned by *Up* and *Down* macrostates (resp), here we plot the microstate transition graph for the $I_2$ macrostate.