We arbitrarily define a water molecule to be in the extracellular vestibule (EV) if its oxygen atom is within 26 A of the CB atom of Phe-326 but not within 5 A of lipid atoms, and its z-coordinate is larger but no more than 23 A above that of the same reference atom (the z-axis is perpendicular to the membrane and points toward the extracellular side), following the method used in Zhao et al. 2012 for LeuT. We divide this vestibule in two different parts: the extracellular cavity (ECav), which includes the water molecules in which its oxygen atom is further than 10 A from the dopamine and the extracellular channel (ECha) with the molecules that are closer.

Similarly, we arbitrarily define a water molecule to be in the intracellular vestibule (IV) if its oxygen atom is within 15 A of the dopamine but again not within 5 A of any lipid, and its z-coordinate is larger but no more than 15.5 A above the CB atom of Asp-436. The division between intracellular cavity (ICav) and intracellular channel (ICha) is given in relation to the backbone of Thr-269. The waters that are further than 12 A to that region are defined to be in ICav and the ones that are closer in ICha.

The clustering of the frames in the trajectories into different groups was done using k-means algorithm. The preprocessing of the data included the “smoothing” of the variables, in which every variable at each timestep takes the value of the average of that variable in the 100 nearest timesteps. The variables used for the clustering were the water in ECav and ECha, the distance between TM1a and TM6b (measured as the distance of the center of mass of the CA in the last three residues of each TM), distance between Na1 and its nearest sodium, the distance between Glu-428 and its nearest cation in the intracellular water and the number of contacts between IL4 and the N-terminus mediated by PIP2 (measured as the number of positive residues of both fragments which CA atoms lie as much as 10 A to each other and with a PIP2 molecule lying closer than 3 A away to those residues).

The correlation among variables was measured by computing the correlation matrix of all the frames, after the smoothing of the variables.