Aquaporins are transmembrane channels found in cell membranes of all life forms. We examine their apparently paradoxical property, facilitation of efficient permeation of water while excluding protons, which is of critical importance to preserving the electrochemical potential across the cell membrane. We have determined the structure of the Escherichia coli aquaglyceroporin GlpF with bound water, in native (2.7 angstroms) and in W48F/F200T mutant (2.1 angstroms) forms, and carried out 12-nanosecond molecular dynamics simulations that define the spatial and temporal probability distribution and orientation of a single file of seven to nine water molecules inside the channel. Two conserved asparagines force a central water molecule to serve strictly as a hydrogen bond donor to its neighboring water molecules. Assisted by the electrostatic potential generated by two half-membrane spanning loops, this dictates opposite orientations of water molecules in the two halves of the channel, and thus prevents the formation of a "proton wire," while permitting rapid water diffusion. Both simulations and observations revealed a more regular distribution of channel water and an increased water permeability for the W48F/F200T mutant.