Supplementary Material

Selectivity mechanism of muscarinic acetylcholine receptors antagonism through *in silico* investigation

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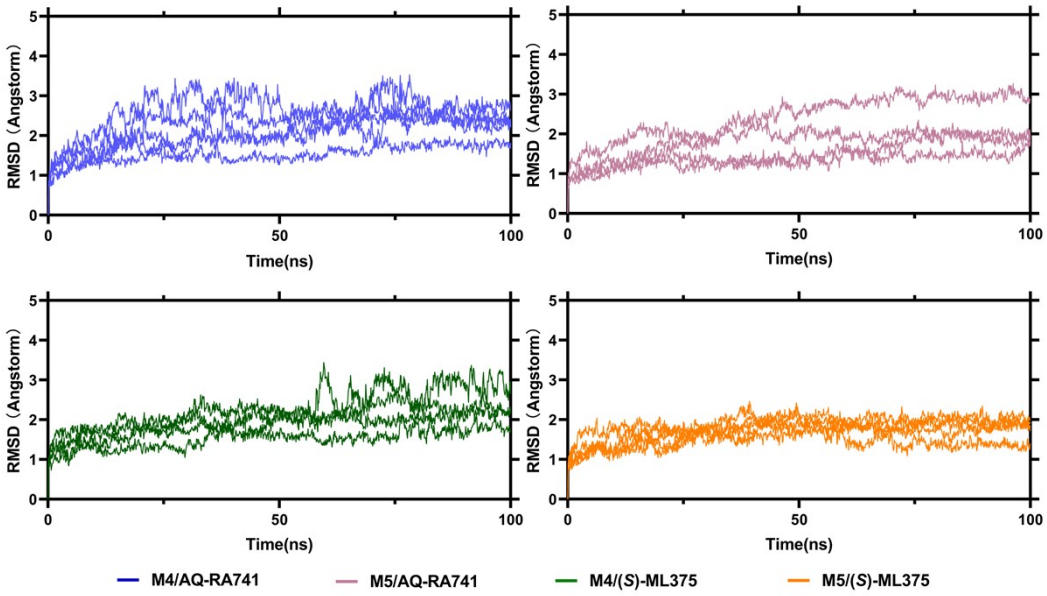

Fig. S1. RMSD curves of M4/5 protein backbone atoms throughout the 100 ns multiple replica molecular dynamics simulations. M4/AQ-RA741 (blue line), M4/(*S*)-ML375 (green line), M5/AQ-RA741 (violet line), M5/(*S*)-ML375 (orange line).

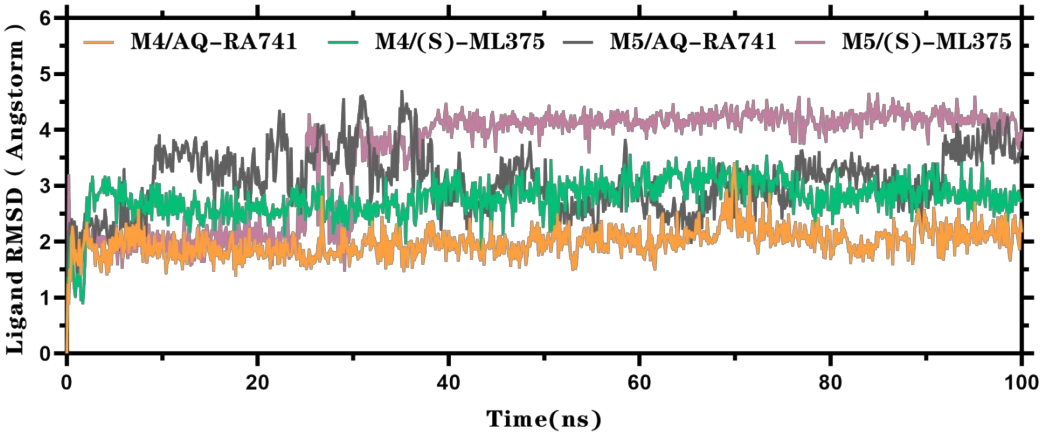

Fig. S2. RMSD plots of ligands throughout the 100 ns MD simulations. M4/AQ-RA741 (orange line), M4/(*S*)-ML375 (green line), M5/AQ-RA741 (gray line), M5/(*S*)-ML375 (violet line).

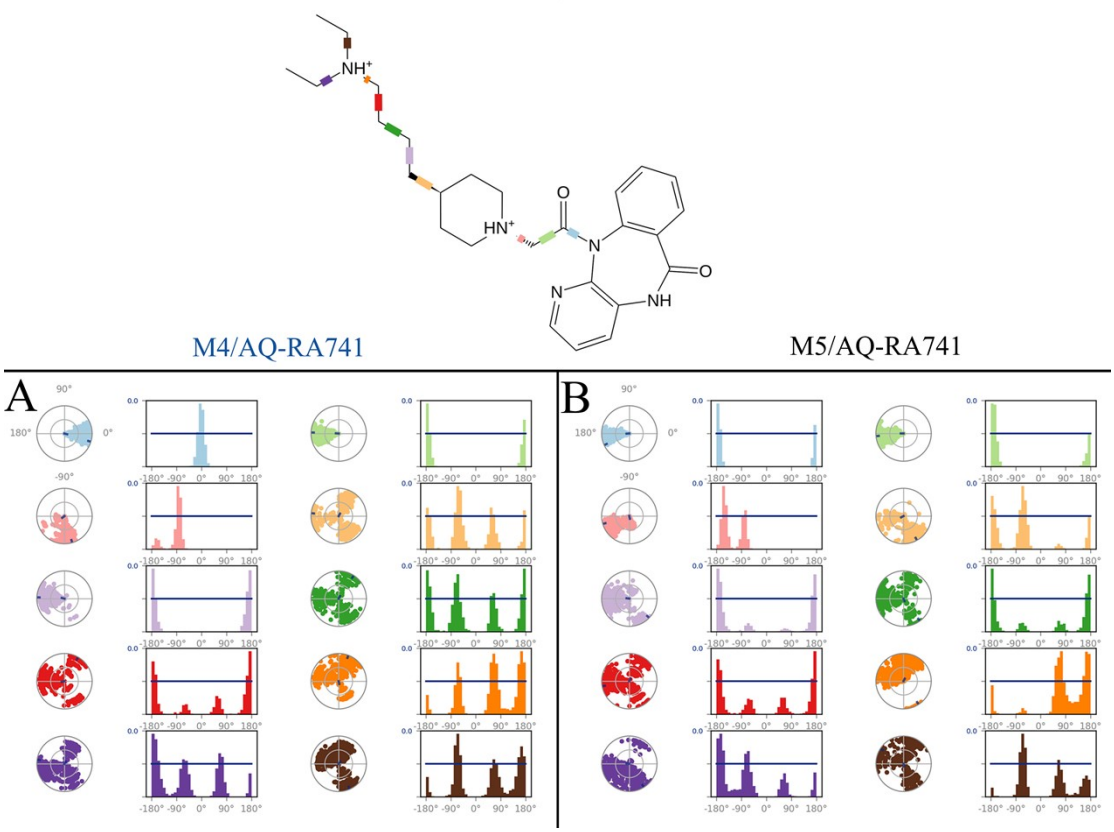

Fig. S3. The ligand torsions plot of AQ-RA741 in complex with M4 and M5 throughout the 100ns simulation trajectory. The left panel shows the 2d schematic of the antagonist AQ-RA741 with color-coded rotatable bonds. Each rotatable bond torsion is accompanied by a dial plot and bar plots of the same color.

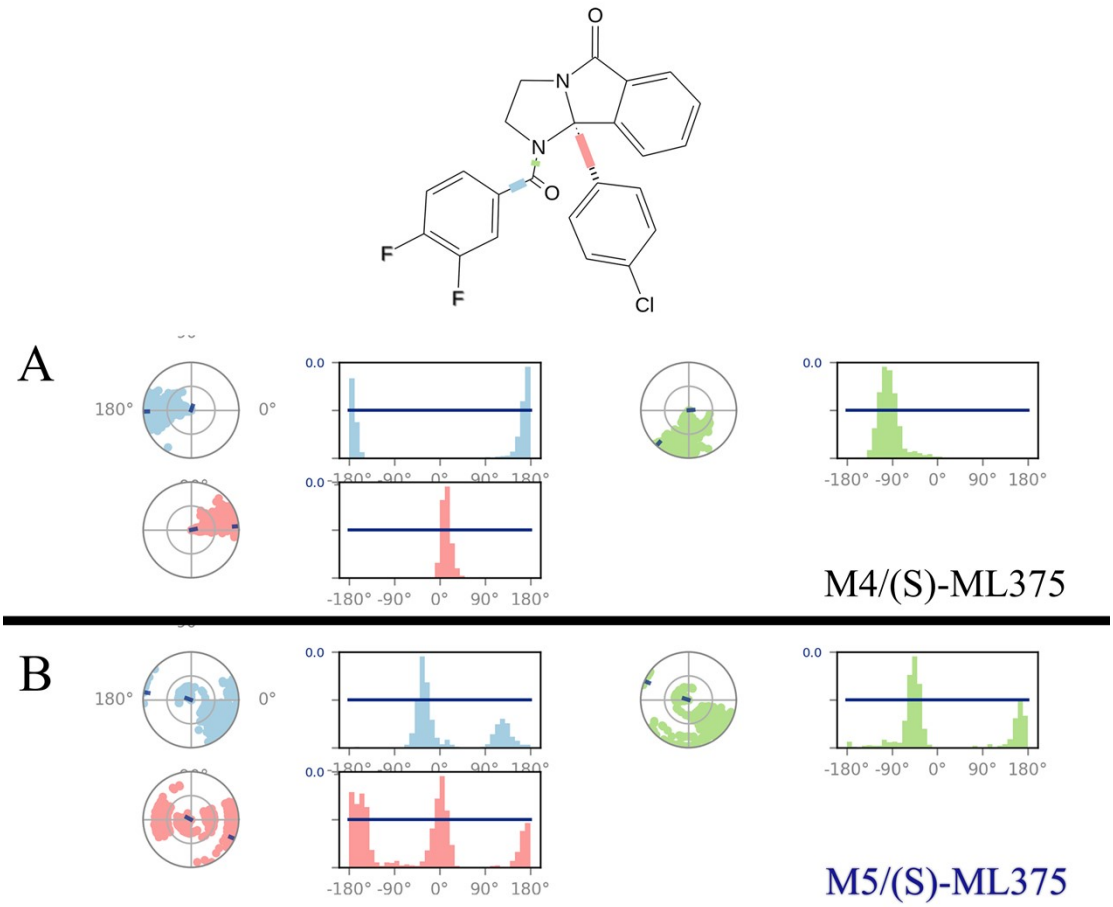

Fig. S4. (A) The ligand torsions plot of M4 during the 100ns MD simulation. (B) The ligand torsions plot of M5 throughout the 100ns simulation trajectory. The left panel shows the 2d schematic of the antagonist (*S*)-ML375 with color-coded rotatable bonds. Each rotatable bond torsion is accompanied by a dial plot and bar plots of the same color.

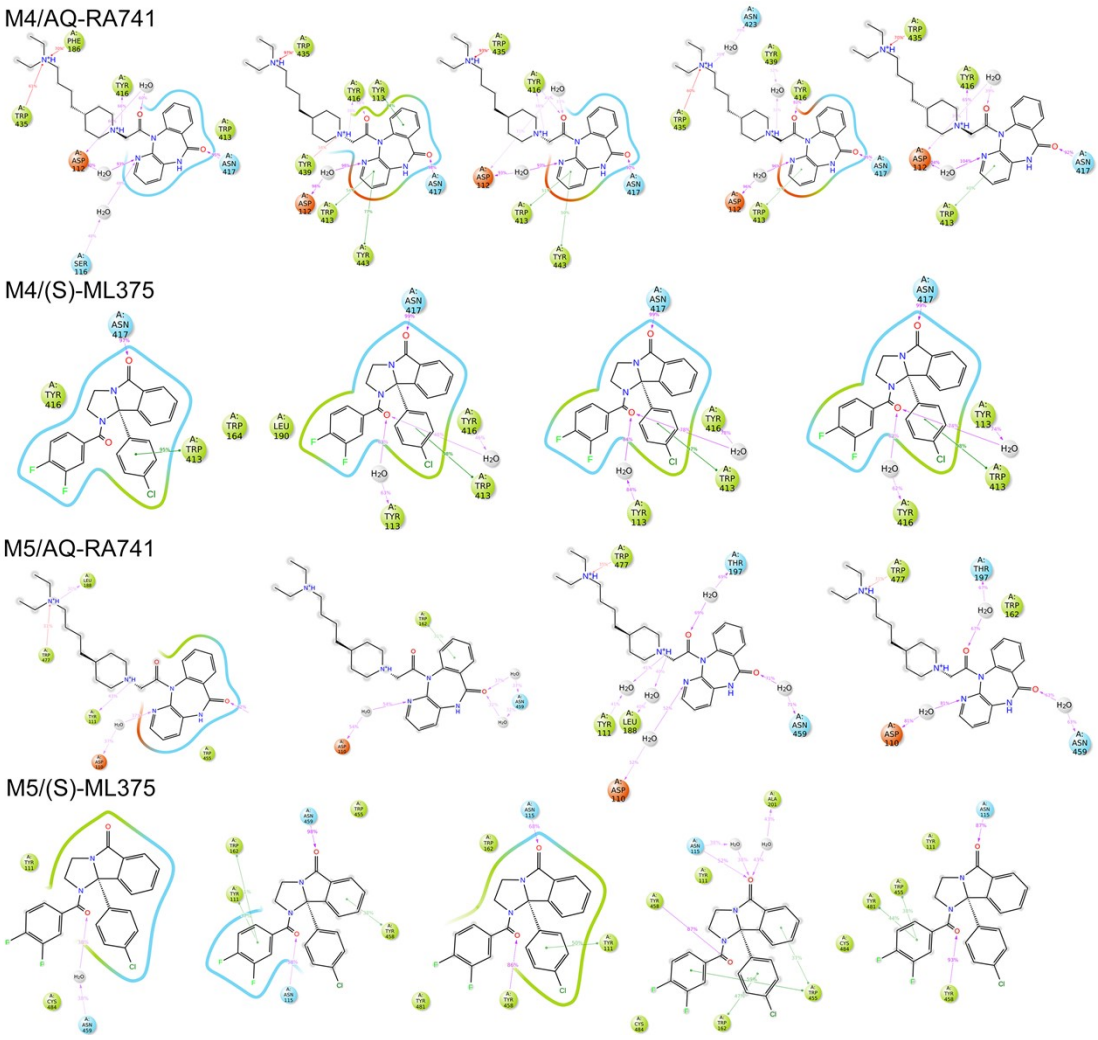

Fig. S5. Molecular interactions of antagonists AQ-RA741/(*S*)-ML375 generated from the multiple replica molecular dynamics simulations.

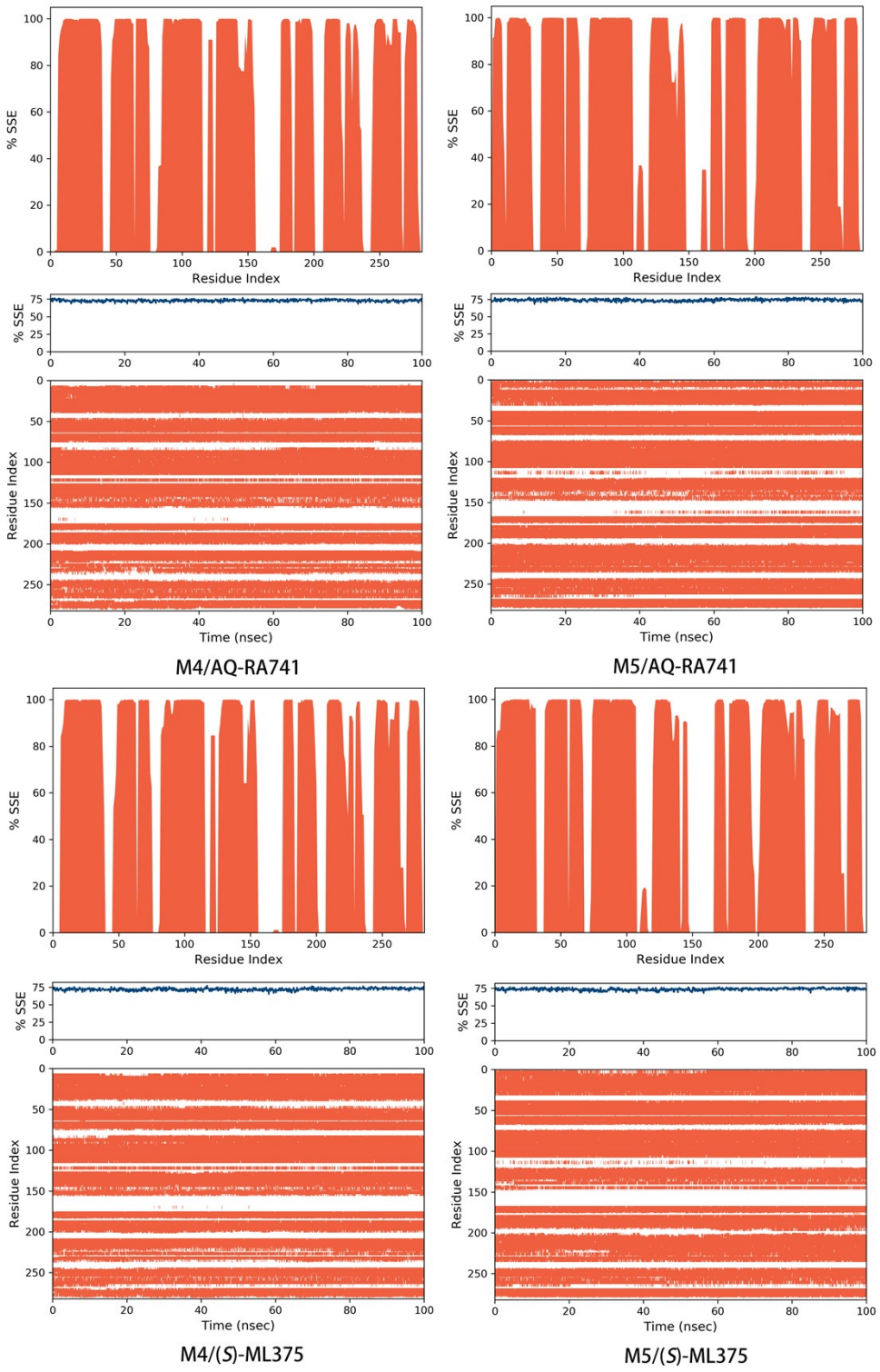

Fig. S6. Protein secondary structure elements (SSE) like alpha-helices and beta-strands are monitored throughout the simulation. The plot above reports SSE distribution by residue index throughout the protein structure. The plot below summarizes the SSE composition for each trajectory frame over the course of the simulation, and the plot at the bottom monitors each residue and its SSE assignment over time.