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Supplemental Information

Identification of Two New Cholesterol Interaction Sites on the A_{2A} Adenosine Receptor

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Supplemental information for Identification of two new cholesterol interaction sites on the A_{2A} adenosine receptor

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Supporting Materials and Methods

Anton2 final.ark file (full simulation configuration)

```
anton {
    chem {
        Escale = "1000"
        Fscale = "1000"
        Tempmax = "350"
        average_dispersion_type = "manual"
        r_over_sigmamin = "0.55"
        rmin = "0.5"
        useries {
            accuracy_level = "-1"
        }
    }
    tune {
        Bumpiness = "1.72362525504993691072"
        Subboxes = ["8" "4" "4"]
        VirtualSubboxes = ["8" "4" "4"]
        checkpoint {
            first = "0"
            minutes = "2"
            on_last_timestep = "true"
            outdir =
"/anton2fs/raw/lymane/prot2_prod/workdir/jobsteps/000020-
00018868/checkpoint.atr"
        }
        development {
            icb {
                max_pos_queue_size = "1024"
            }
        }
        energy {
            format = "etr"
            interval = "240"
            outdir =
"/anton2fs/raw/lymane/prot2_prod/workdir/jobsteps/000020-
00018868/energy.etr"
        }
        engine {
            derived {
                double_buffer_htis_queues = "true"
            }
            migration {
                channels = "16"
                interval = "9"
```

```
                offset = "-1"
            }
        }
        htis {
            Tiles = ["1" "2" "1"]
            cs {
                StoredSetReplication = "1"
                Subboxing = ["1" "1" "1"]
            }
            fi {
                StoredSetReplication = "1"
                Subboxing = ["2" "1" "1"]
            }
            mid {
                StoredSetReplication = "1"
                Subboxing = ["2" "1" "1"]
            }
        }
        jobstep_wallclock = "60.0"
        last_time = "6000000"
        machine_size = ["4" "4" "8"]
        max_strain = "0.1"
        optional {
            bootframeset =
"/anton2fs/raw/lymane/prot2_prod/workdir/jobsteps/000019-
00018817/checkpoint.atr"
            bootframeset_neartime = "5799840.0"
            strain_free_system_size = ["154.79" "138.75" "121.38"]
        }
        trajectory {
            interval = "240"
            mb_per_file = "100"
            outdir =
"/anton2fs/raw/lymane/prot2_prod/workdir/jobsteps/000020-
00018868/run.dtr"
        }
    }
}
boot {
    file = "/anton2fs/raw/lymane/prot2_prod/workdir/prot2_wff.dms"
}
force {
    nonbonded {
        average_dispersion = "0"
        electrostatic {
            type = "useries"
        }
        far {
            n_k = ["64" "64" "64"]
            r_spread = "9.05629019737243545762"
            sigma_s = "2.09060048085864913503"
        }
        r_cut = "9.0572901973724349034"
        type = "vdw-elec"
        vdw_r_cut = "9"
```

```
    }
}
integrator {
    Multigrator {
        barostat {
            MTK {
                T_ref = "295.00"
                tau = "0.0416667"
                thermostat {
                    NoseHoover {
                        chain {
                            mts = "4"
                            tau = ["0.0416667" "0.0416667"
"0.0416667"]
                        }
                    }
                    type = "NoseHoover"
                }
            }
            interval = "480"
            type = "MTK"
        }
        nve {
            type = "Verlet"
        }
        thermostat {
            Antithetic {
                use_molecular_ke = "true"
            }
            NoseHoover {
                chains = [{
                    mts = "1"
                    tau = [".0416667" ".0416667" ".0416667"]
                }]
                use_molecular_ke = "true"
            }
            interval = "24"
            type = "NoseHoover"
        }
    }
    dt = "0.0025"
    pressure {
        isotropy = "semi_isotropic"
        p_ref = "1.0"
        tension_ref = ["0." "0." "0." "0." "0." "0." "0." "0." "0."]
    }
    remove_com_motion = "true"
    respa {
        bonded_interval = "1"
        nonbonded_far_interval = "3"
        nonbonded_near_interval = "1"
    }
    temperature = [{
        T_ref = "295.00"
    }]
```

```
    type = "Multigrator"
}
jobengine {
    local_preprep = "1"
    skip_preprep = "0"
}
```

Supporting Results

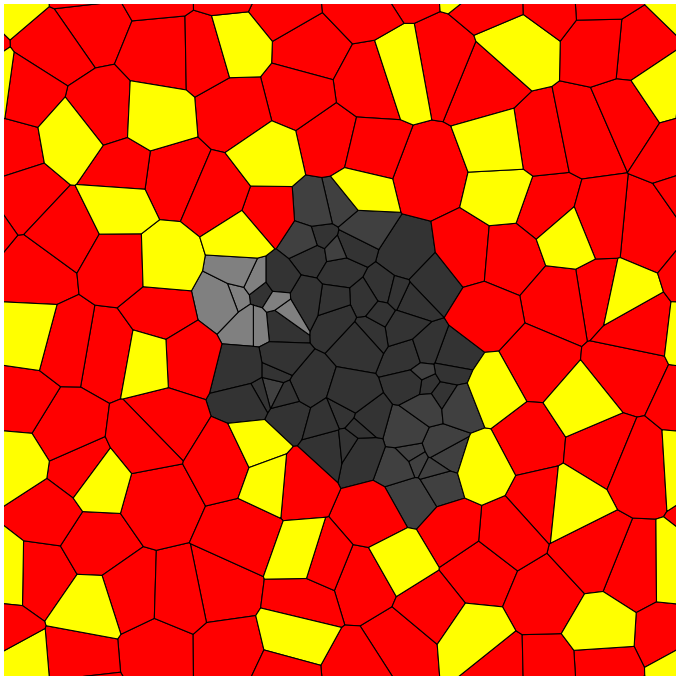

Figure S1. Example Voronoi construction for one leaflet, showing POPC (red) and cholesterol (yellow) around $A_{2A}R$ (gray).

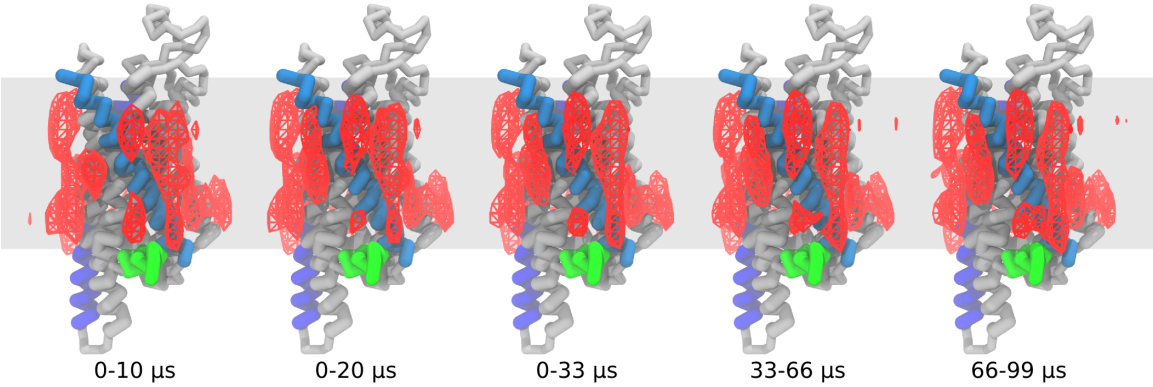

Figure S2. Convergence of cholesterol densities during 100 μsec Martini simuation. Wireframe indicates cholesterol density at least 10x the bulk density.

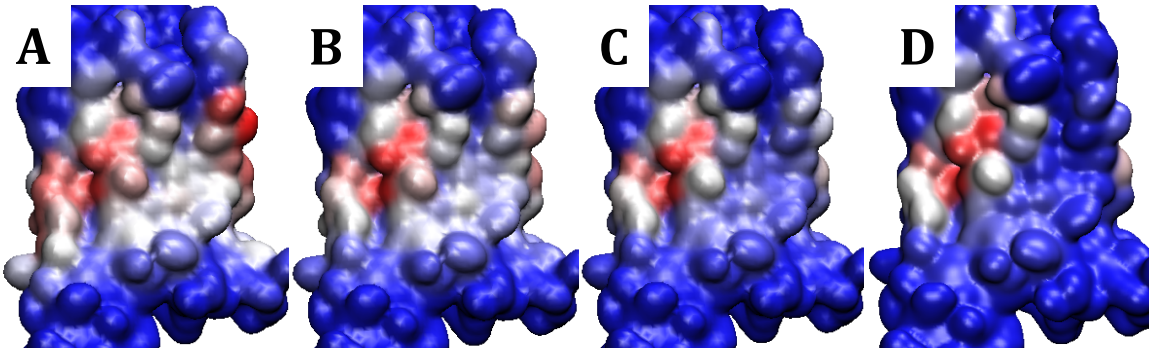

Figure S3. Residue ranking is insensitive to the definition of the interaction timescale, provided it is at least 500 nsec. The heatmap showing the score of each residue is shown for four choices of the cutoff time for defining an interaction event: 100 nsec (panel A), 500 nsec (panel B), 1,000 nsec (panel C), 2,000 nsec (panel D).

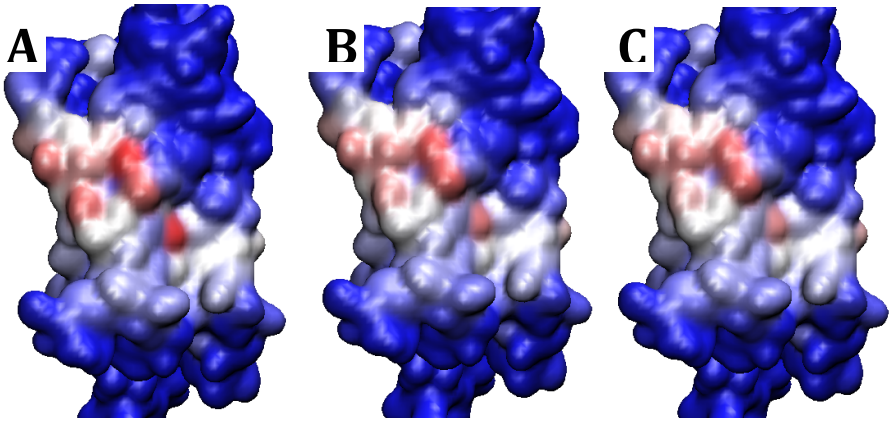

Figure S4. Residue ranking is insensitive to the definition of the cutoff distance for defining a cholesterol/protein contact. The heatmap showing the score of each residue is shown for three choices of the cutoff distance for defining an interaction event: 0.55 nm (panel A), 0.65 nm (panel B), 0.70 nm (panel C).

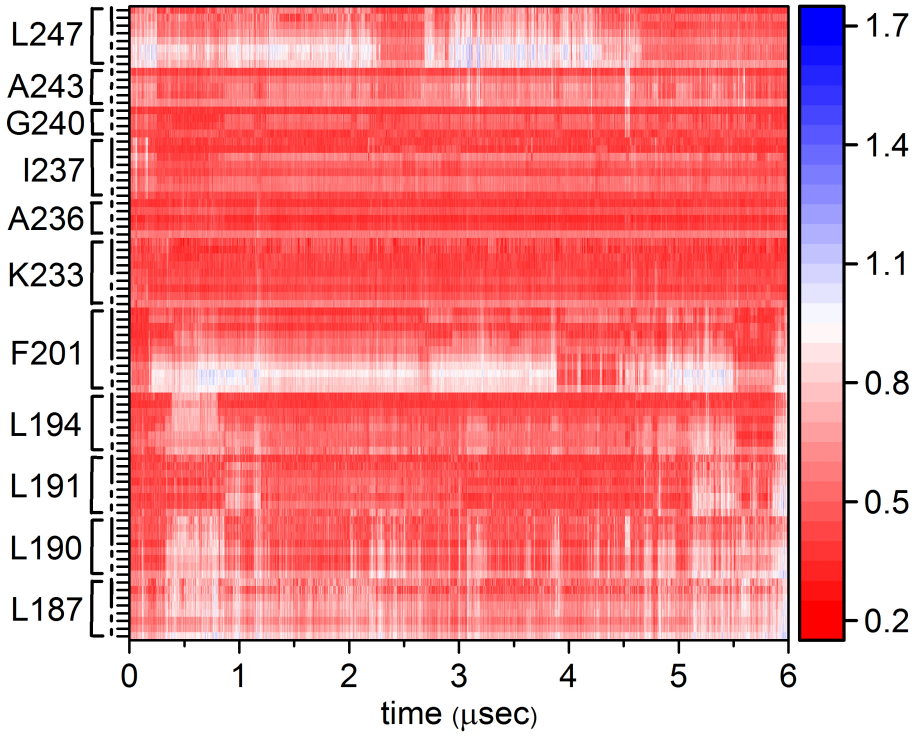

Figure S5. Interaction of cholesterol with h56i in all atom simulation of ZM241385 bound receptor. The distance between each heavy atom of selected sidechains and the closest atom of any cholesterol (in nm). Though many cholesterols make transient contacts with different residues, in this trajectory a single cholesterol remains bound throughout the entire trajectory. The sequence is indicated at the left, the protein backbone is indicated by the dashed line, the sidechains by the solid vertical lines.

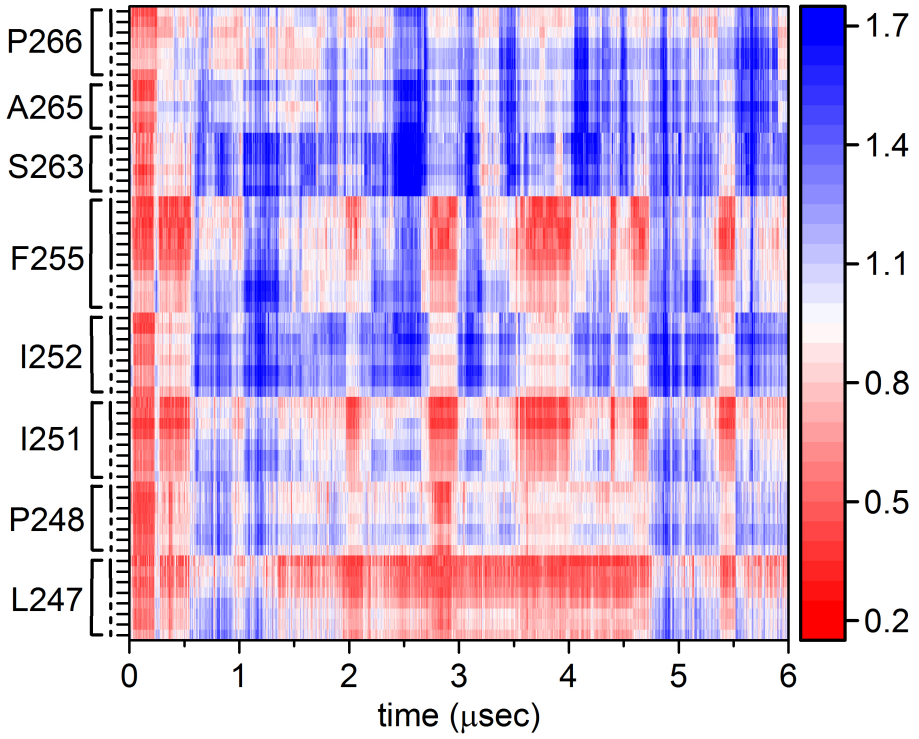

Figure S6. Interaction of cholesterol with h6o in all atom simulation of the apo receptor. The distance between each heavy atom of selected sidechains and the closest atom of any cholesterol (in nm). Many cholesterols make transient contacts with different residues, in this trajectory the only binding event occurs during the initial 500 nsec of the simulation. The sequence is indicated at the left, the protein backbone is indicated by the dashed line, the sidechains by the solid vertical lines.

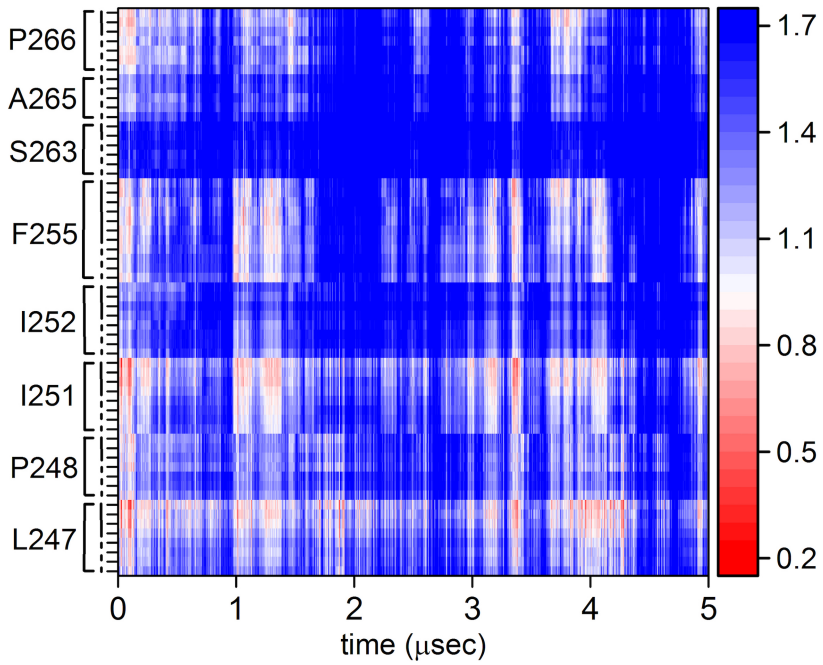

Figure S7. Interaction of cholesterol with h6o in all atom simulation of the NECA bound receptor. The distance between each heavy atom of selected sidechains and the closest atom of any cholesterol (in nm). Many cholesterols make transient contacts with different residues, in this trajectory the only binding event occurs during the initial 500 nsec of the simulation. The sequence is indicated at the left, the protein backbone is indicated by the dashed line, the sidechains by the solid vertical lines.

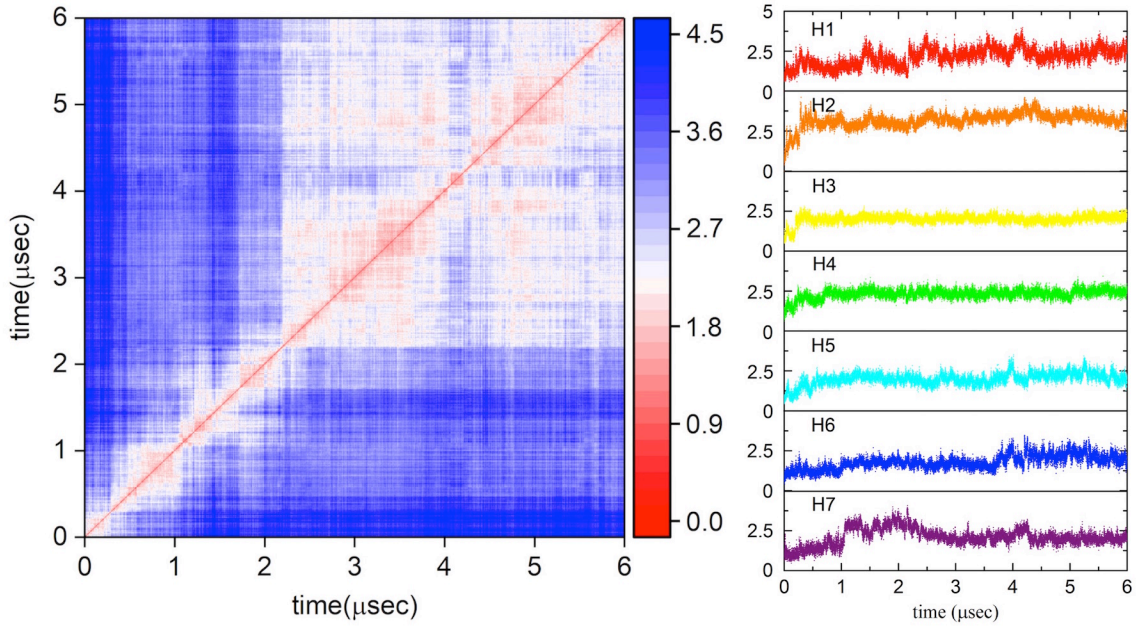

Figure S8. Conformational landscape of apo simulation. The left panel shows the RMSD matrix of the all atom apo simulation. The right panel shows the RMSD of each of the transmembrane helices. The entire transmembrane bundle is used as a reference for alignment in the left panel, so that relative motion of helices is captured.

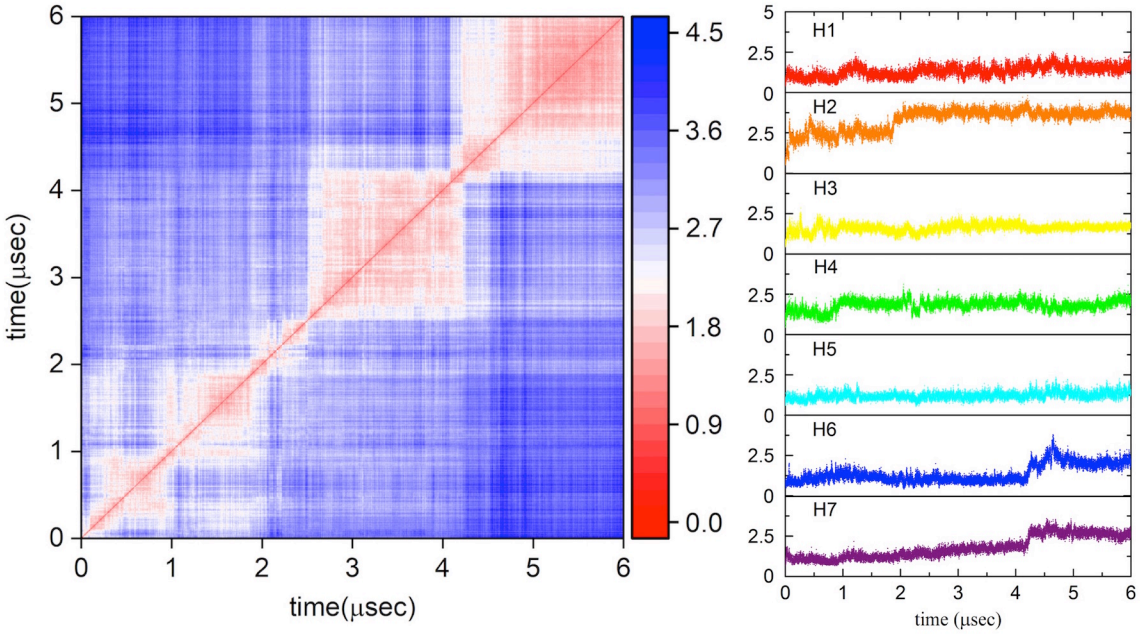

Figure S9. Conformational landscape of the ZM241385 bound simulation. The left panel shows the RMSD matrix (backbone heavy atoms) of the all atom ZM241385 bound simulation. The right panel shows the RMSD (backbone heavy atoms) of each of the transmembrane helices. The entire transmembrane bundle is used as a reference for alignment in the left panel, so that relative motion of helices is captured.

Movie M1. 200 nsec portion of the all-atom simulation of the apo receptor, showing cholesterol bound to h56i. See caption of Fig. 5 for explanation of rendering scheme.

Movie M2. 200 nsec portion of the all-atom simulation of the ZM241385-bound receptor, showing cholesterol bound to h6o. See caption of Fig. 6 for explanation of rendering scheme.