Distinct binding of cetirizine enantiomers to human serum albumin and the human histamine receptor H_1

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Supplementary Information

Table 1. Data collection and refinement statistics for the ESA:CTZ complex.

Resolution range	47.12 - 2.151 (2.228 - 2.151)
Space group	P 61
Unit cell	94.234 94.234 141.893 90 90 120
Unique reflections	38630 (3823)
Completeness (%)	99.81 (99.43)
Wilson B-factor	44.72
Reflections used in refinement	38630 (3823)
Reflections used for R-free	1879 (161)
R-work	0.1903 (0.2500)
R-free	0.2470 (0.2970)
Number of non-hydrogen atoms	4779
macromolecules	4490
ligands	182
solvent	107
Protein residues	580
RMS (bonds)	0.007
RMS (angles)	1.15
Ramachandran favored (%)	97.40
Ramachandran allowed (%)	2.60
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	3.35
Clashscore	6.03
Average B-factor	57.52
macromolecules	56.73
ligands	80.27
solvent	52.11

Statistics for the highest-resolution shell are shown in parentheses.

Fig. S1 Pairwise structural alignment (Dali) [1] of ESA (PDB entry **5dqf**) to HSA (PDB entry **1e7c**).[a]

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                    :         :         :         :         |         :         :         :         :        102
0001 1e7cA  HKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECF
0002 5dqfA  -KSEIAHRFNDLGEKHFKGLVLVAFSQYLQQCPFEDHVKLVNEVTEFAKKCAADEsaencdksLHTLFGDKlctvatlratygeladccekqepernecf
                    :         :         :         :         |         :         :         :         :        102
0001 1e7cA  LLLHHHHHHHHHLHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHHHLLLLLLLLLLHHHHHHHHHLLLLLHHHHHHHHHHHHLHHHHHHHH
0002 5dqfA  -LLHHHHHHHHHLHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHHHLLlllllllHHHHHHHHhhllllhhhhlhhhhhhllllllhhhhhh

                    :         :         :         :         |         :         :         :         :        202
0001 1e7cA  LQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCAS
0002 5dqfA  ltHKDDHPNLPKL-KPEPDAQCAAFQEDPDKFLGKYLYEVARRHPYFYGPELLFHAEEYKADFTECCPADDKLACLIPKLDALKERILLSSAKERLKCSS
                    :         :         :         :         |         :         :         :         :        201
0001 1e7cA  HHLLLLLLLLLLLLLLLHHHHHHHHHHLHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHHHHHHHH
0002 5dqfA  hhLLLLLLLLLLL-LLLHHHHHHHHHHLHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHHHHHHH

                    :         :         :         :         |         :         :         :         :        302
0001 1e7cA  LQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADL
0002 5dqfA  FQNFGERAVKAWSVARLSQKFPKADFAEVSKIVTDLTKVHKECCHGDLLECADDRADLAKYICEHQDSISGKLKACCDKPLLQKSHCIAEVKEDDLPSDL
                    :         :         :         :         |         :         :         :         :        301
0001 1e7cA  HHHHHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHHLLHHHHHHHHHHHHHHHHHHLHHHLLLLLHHHHHLLHHHHHHHHHHLLLLLLLLLL
0002 5dqfA  HHHHLHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHHLLLHHHHHHHHHHHHHHHHHHLHHHHLHHHHHHHLLLHHHHHHHHHLLLLLLLLLL

                    :         :         :         :         |         :         :         :         :        402
0001 1e7cA  PSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYK
0002 5dqfA  PALAADFAEDKEICKHYKDAKDVFLGTFLYEYSRRHPDYSVSLLLRIAKTYEATLEKCCAEADPPACYRTVFDQFTPLVEEPKSLVKKNCDLFEEVGEYD
                    :         :         :         :         |         :         :         :         :        401
0001 1e7cA  LLLHHHHLLLLLHHHHHHHLHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLLHHHLLLLLHHHHHHHHHHHHHHHHHHHHHLHHH
0002 5dqfA  LLLHHHHLLLLLHHHHHHHLHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLLHHHHLLHHHHHHHHHHHHHHHHHHHHHHHHH

                    :         :         :         :         |         :         :         :         :        502
0001 1e7cA  FQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF
0002 5dqfA  FQNALIVRYTKKAPQVSTPTLVEIGRTLGKVGSRCCKLPESERLPCSENHLALALNRLCVLHEKTPVSEKITKCCTDSLAERRPCFSALELDEGYVPKEF
                    :         :         :         :         |         :         :         :         :        501
0001 1e7cA  HHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLHHHHHHHHHHHHHHHHHHHHHHHHHHHHLLLLHHHHHHHHLLLLLHHHHHHHLLLLLLLLLLL
0002 5dqfA  HHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLHHHHHHHHHHHHHHHHHHHHHHHHHHHHLLLLHHHHHHHHLLLLLHHHHHHHLLLLLLLLLLL

                    :         :         :         :         |         :         :         : 584
0001 1e7cA  NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALG
0002 5dqfA  KAETFTFHADICTLPEDEKQIKKQSALAELVKHKPKATKEQLKTVLGNFSAFVAKCCGAEDKEACFAEEGPKLVASSQLALA
                    :         :         :         :         |         :         :         : 583
0001 1e7cA  LHHHHLLLLLHHHLLHHHHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHLL
0002 5dqfA  LHHHHLLLHHHHHLLHHHHHHHHHHHHHHHHHHHLLLLLHHHHHHHHHHHHHHHHHHHLLLLHHHHHHHHHHHHHHHHLL
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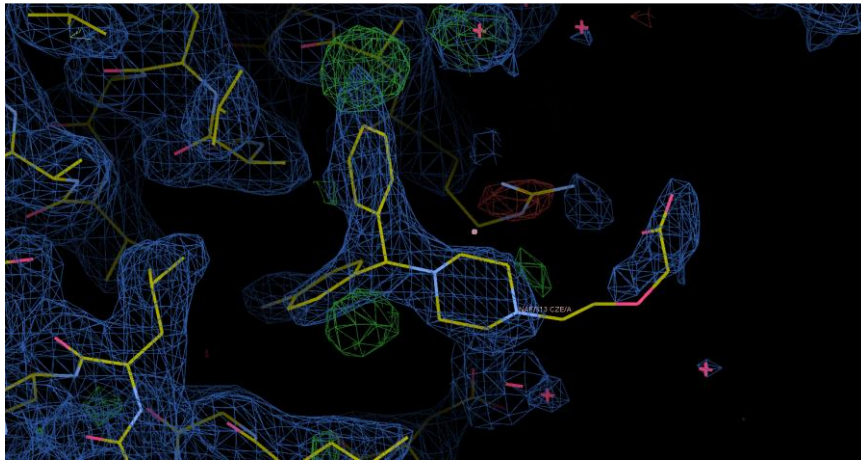
[a] The UniProtKB entries for ESA and HSA are F7BAY6_HORSE and Q56G89_HUMAN, respectively. Uppercase means structurally equivalent positions with HSA; lowercase means insertions relative to HSA. Secondary structure assignments by DSSP (H/h: helix, E/e: strand, L/l: coil). The gap position is highlighted in yellow. Amino acids making up the CBS1 and CBS2 pockets in ESA are colored in red and blue, respectively.

Fig. S2 Detail of the cetirizine-binding sites in the ESA:cetirizine crystallographic complex, as deposited in the PDB with code 5DQF. The $2F_o$-F_c (blue) and F_o-F_c (green) maps are contoured at 1σ and +3σ, respectively, using Coot [2]. Note the unmodeled green blobs next to both ligands.

a CZE (*S*)-cetirizine



b LCR (*R*)-cetirizine

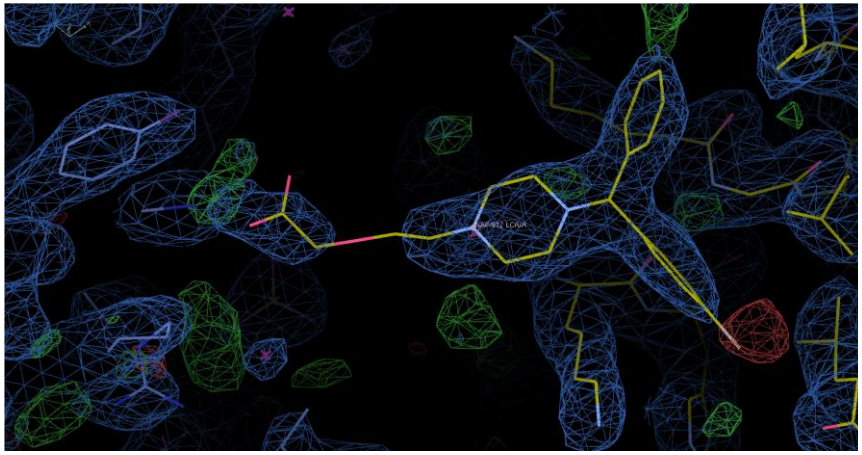
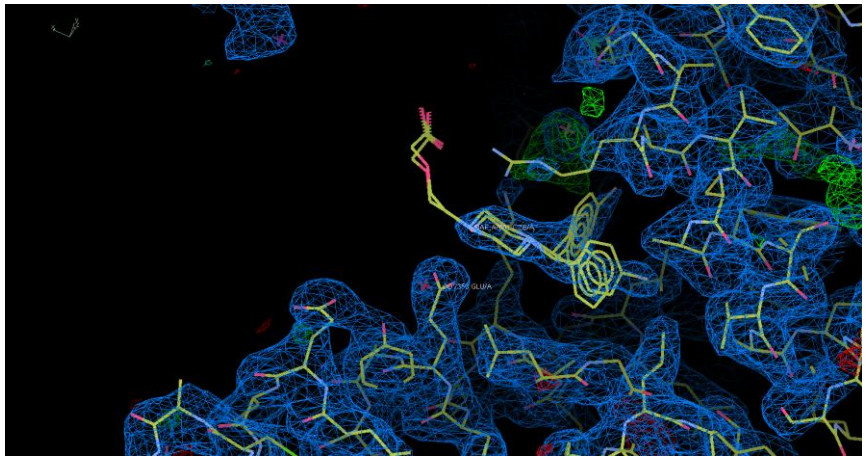


Fig. S3 Detail of the cetirizine-binding site in the ESA:cetirizine crystallographic complex after re-refinement using as initial data the coordinates and structure factors deposited in the PDB with code 5DQF but defining dual occupancies for the two drug enantiomers in both sites. The $2F_o$-F_c (blue) and F_o-F_c (green) maps are contoured at 1σ and +3σ, respectively, using Coot [2]. Note the absence of electron density for the ethoxylacetate moieties of the bound drugs.

a



b

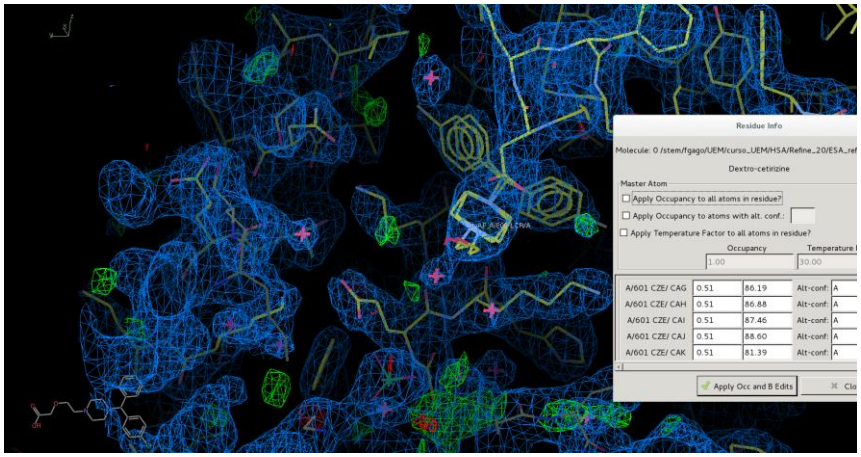


Fig. S4 *h*H_1R model (encompassing amino acids Met28–Cys221 and Leu405–Ile485) used in the simulation. The Cα trace is displayed as a ribbon that has been rainbow-colored to highlight the N-terminus (blue) and C-terminus (red), including α-helix VIII. The bilayer representing the membrane is made up of 240 palmitoyloleoylphosphatidyl-ethanolamine (POPE, C atoms in pink) and 8 cholesterol (C atoms in green) molecules. Sodium and chloride ions in the top and bottom water (aquamarine color) layers are shown as spheres colored in violet and green, respectively.

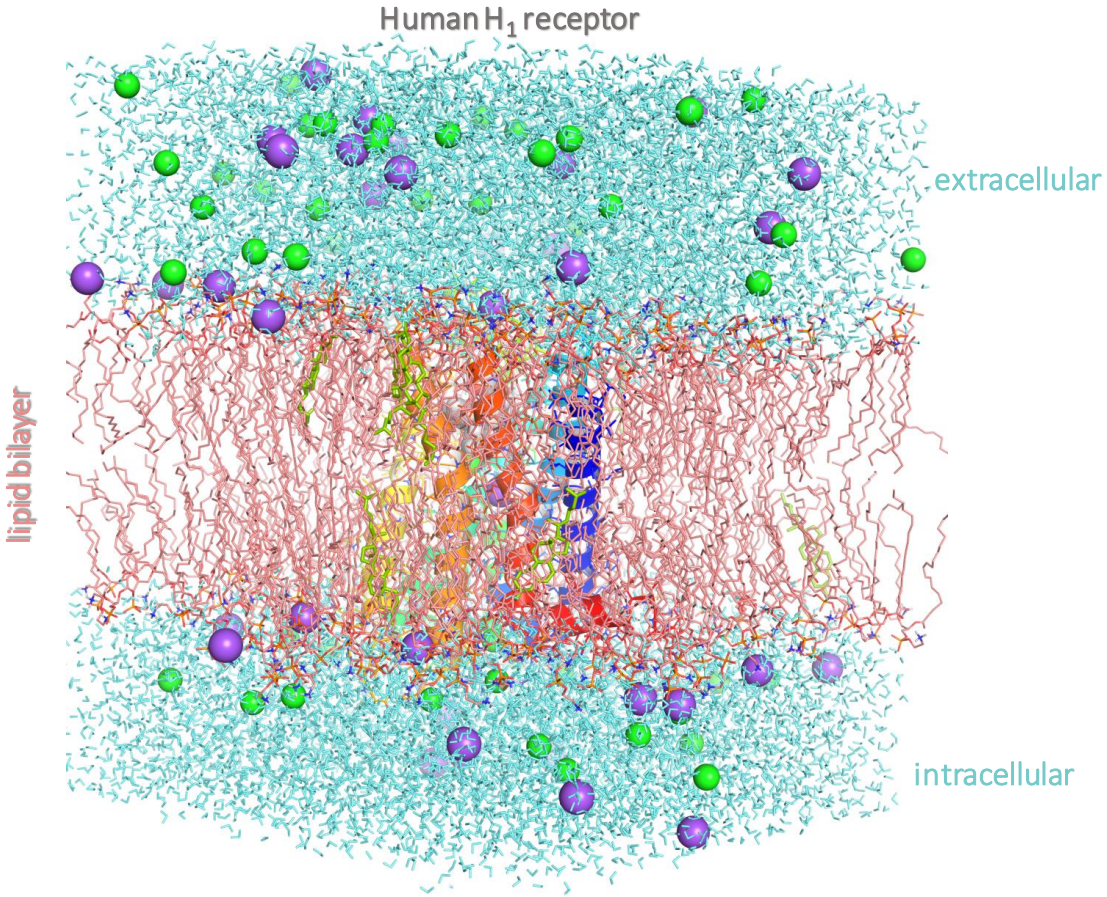


Fig. S5 Schematic of the simulated annealing and energy minimization procedure.

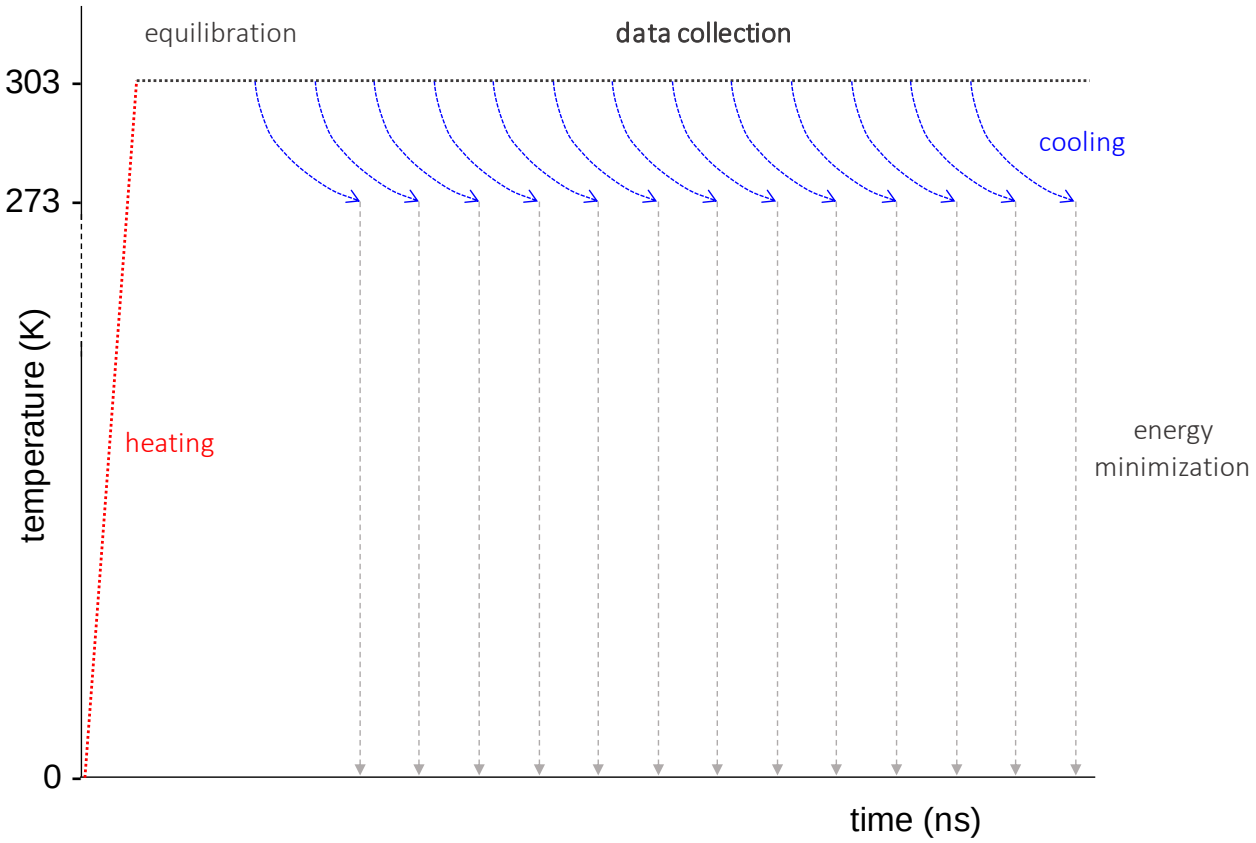
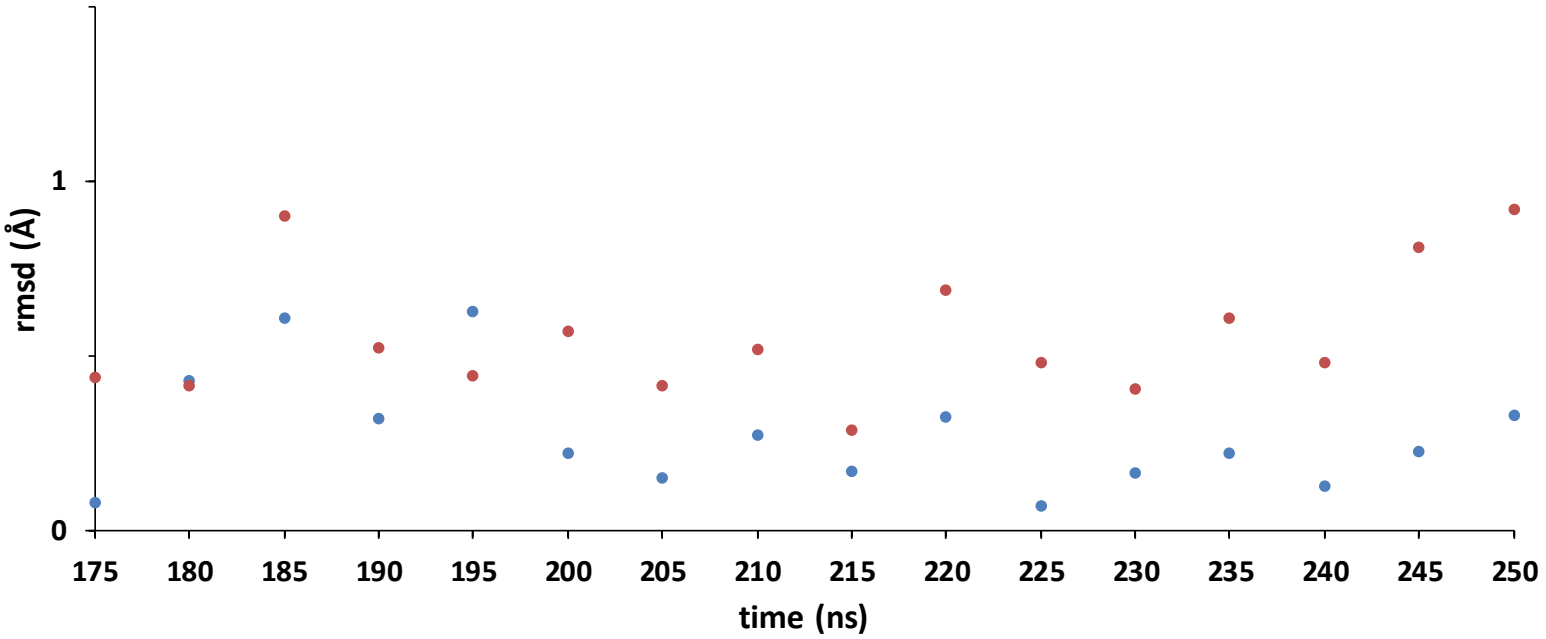


Fig. S6 Time evolution over the course of the uMD trajectories of the root-mean-square deviation (rmsd, Å) of the internally bound Na^+ ion in the *h*H_1R:(*R*)-cetirizine (blue) and *h*H_1R:(*S*)-cetirizine (red) complexes.



References

1. Holm L (2020) Using Dali for protein structure comparison. Methods Mol Biol 2112:29-42. doi:10.1007/978-1-0716-0270-6_3

2. Emsley P, Lohkamp B, Scott WG, Cowtan K (2010) Features and development of Coot. Acta Crystallogr D Biol Crystallogr 66 (Pt 4):486-501. doi:10.1107/S0907444910007493