Supporting Information for

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Binding to the Open Conformation of HIV-1 Protease

Structural Analysis

It is important to determine the root-mean-square deviation of the protein core over the course of simulation. Since the flaps that cover the active site of HIVp are highly flexible during simulation, for our analysis the protease core residues were considered to be all residues except the flaps. Data from our MD simulations was used to calculate the change in RMSD of the Cα backbone with respect to time. Typically, a simulation of stable, folded protein will only diverge from the crystal structure position by 2-4 Å.

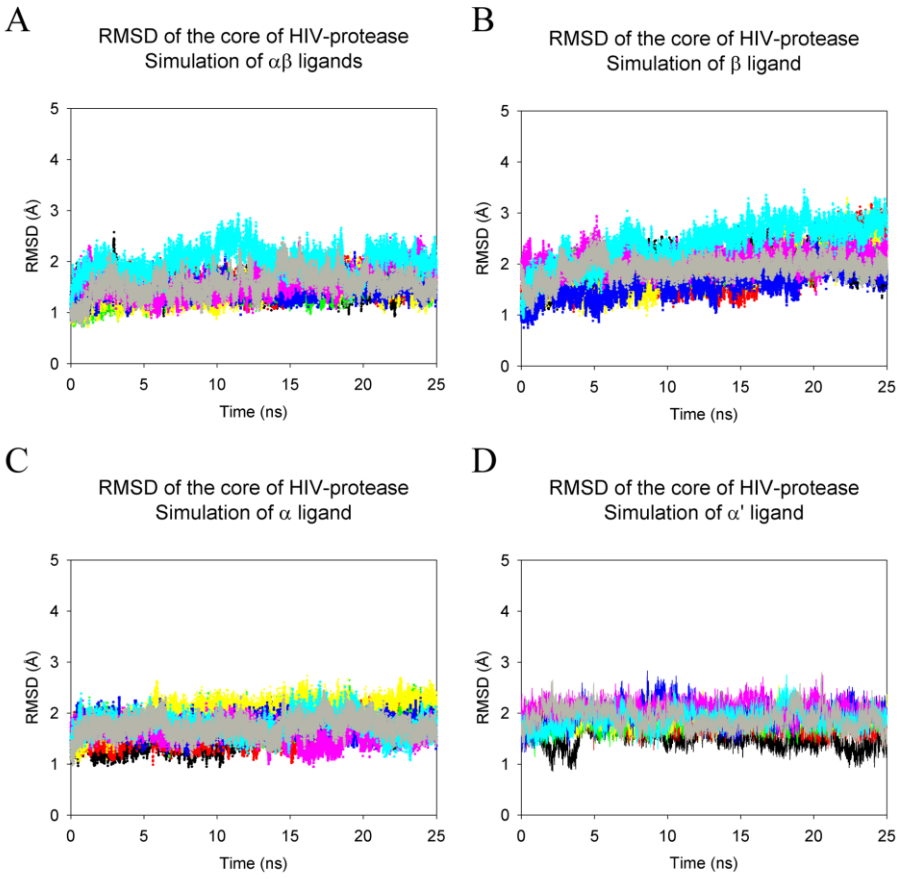

Figure S1: The overall RMSD calculated for the core (all residues but the flaps 43-58/43'-58') of HIVp over the length of the production run. The core remains stable for the duration of the trajecories for each protein-ligand complex αβ (A), β (B), α (C), α' (D).

There are several different ways to measure flap opening in HIVp. One of the most common metrics is the distance from the base of the active site (Asp25/25') to the flap tip (Ile50/50'). The larger the distance, the more open the flaps are considered. A distance of approximately 14 Å is considered closed, while a distance of approximately 18 Å is considered semi-open. An additional metric commonly utilized to measure the extent of flap opening includes the flap tip distance (Ile50-Ile50'). However, due to the nature of a three-dimensional system, this distance measure is heavily influenced by flap curling, which does not necessarily indicate a change in flap conformation. Alternatively, we examined the distance between the Cα atoms' center of mass for the flap residues 48-53/48'-53'. In addition, it was possible to measure the distance from the flap tip to the 80s loop (Ile50-Thr81). This distance can give some insight into the handedness of the flap conformation. A distance of approximately 10 Å is seen in the semi-open structure of 1HHP, while a distance of 15 Å is seen for the bound conformation of 3BC4 as well as the wide-open structure 1TW7. Thus, a distance below 10 Å may indicate a semi-open handedness, while a distance higher than 15 Å may be indicative of a closed handedness.

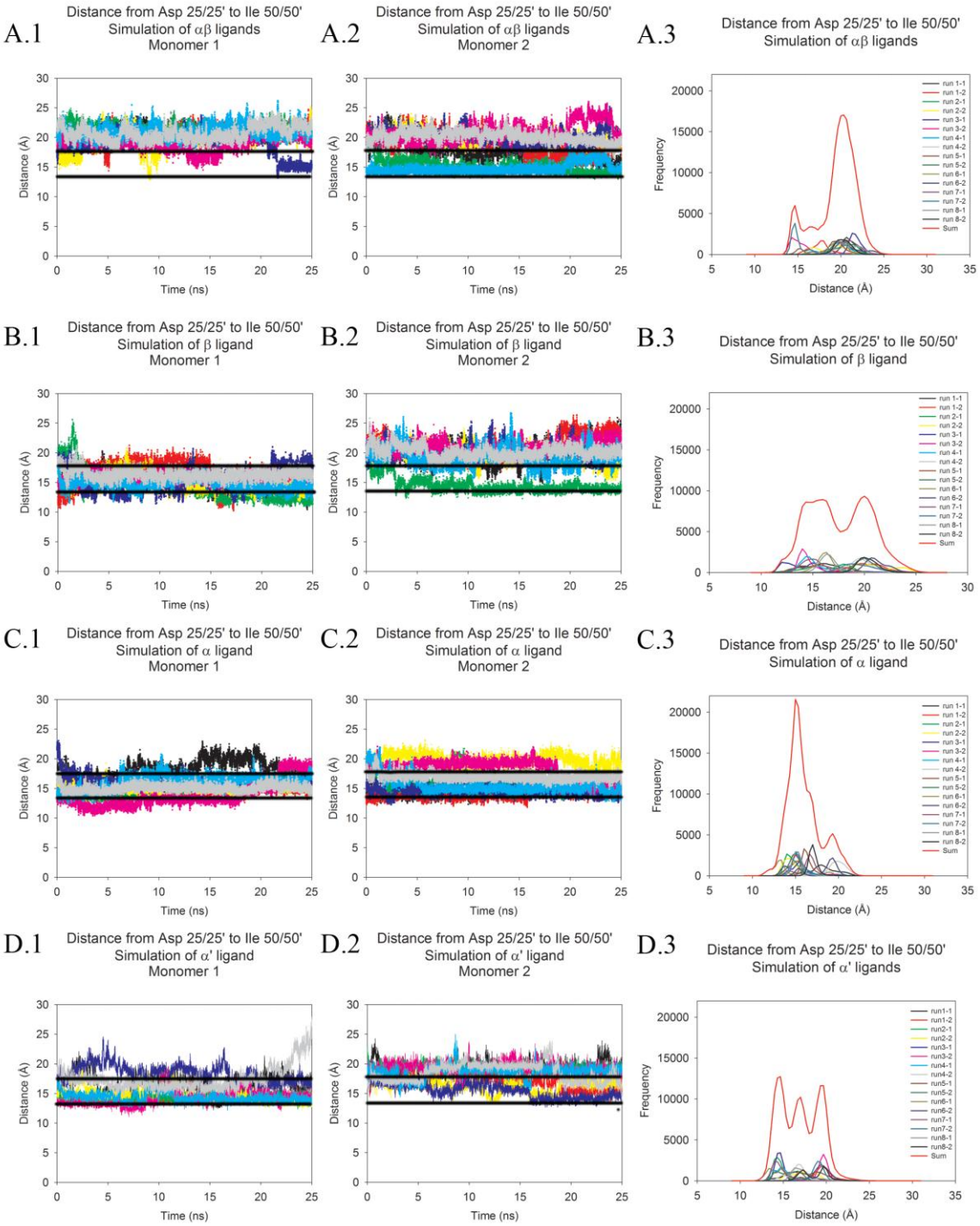

Figure S2: The distance between the catalytic Asp25 and the flap tip Ile50 for monomers 1 and 2 of HIVp over the length of the production runs. The flap-to-active-site distances indicate a wide range of motion for both monomers (1-2), with one majority flap conformation seen for simulations of αβ (A) and α (C), while β (B) and α' (D) are characterized by several equally population flap conformations. The typical closed (14 Å) and semi-open (18 Å) distances are indicated with a black line.

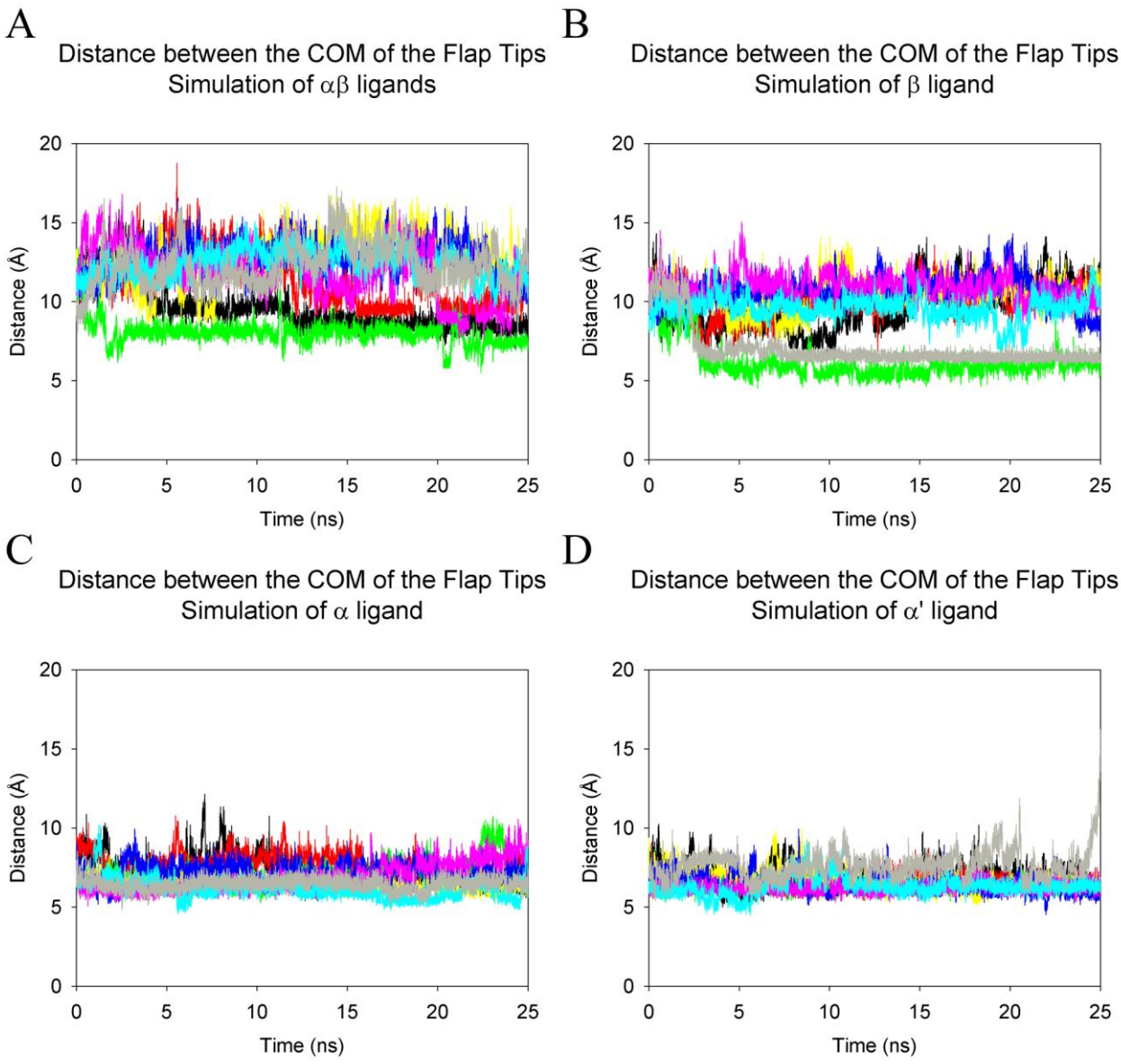

Figure S3: The distance calculated between the center of mass (COM) of the two flaps 43-58/43'-58' over the length of the production run. The flaps are less stable for the duration of the trajecories for complexes αβ (A), β (B), as compared to α (C), α' (D).

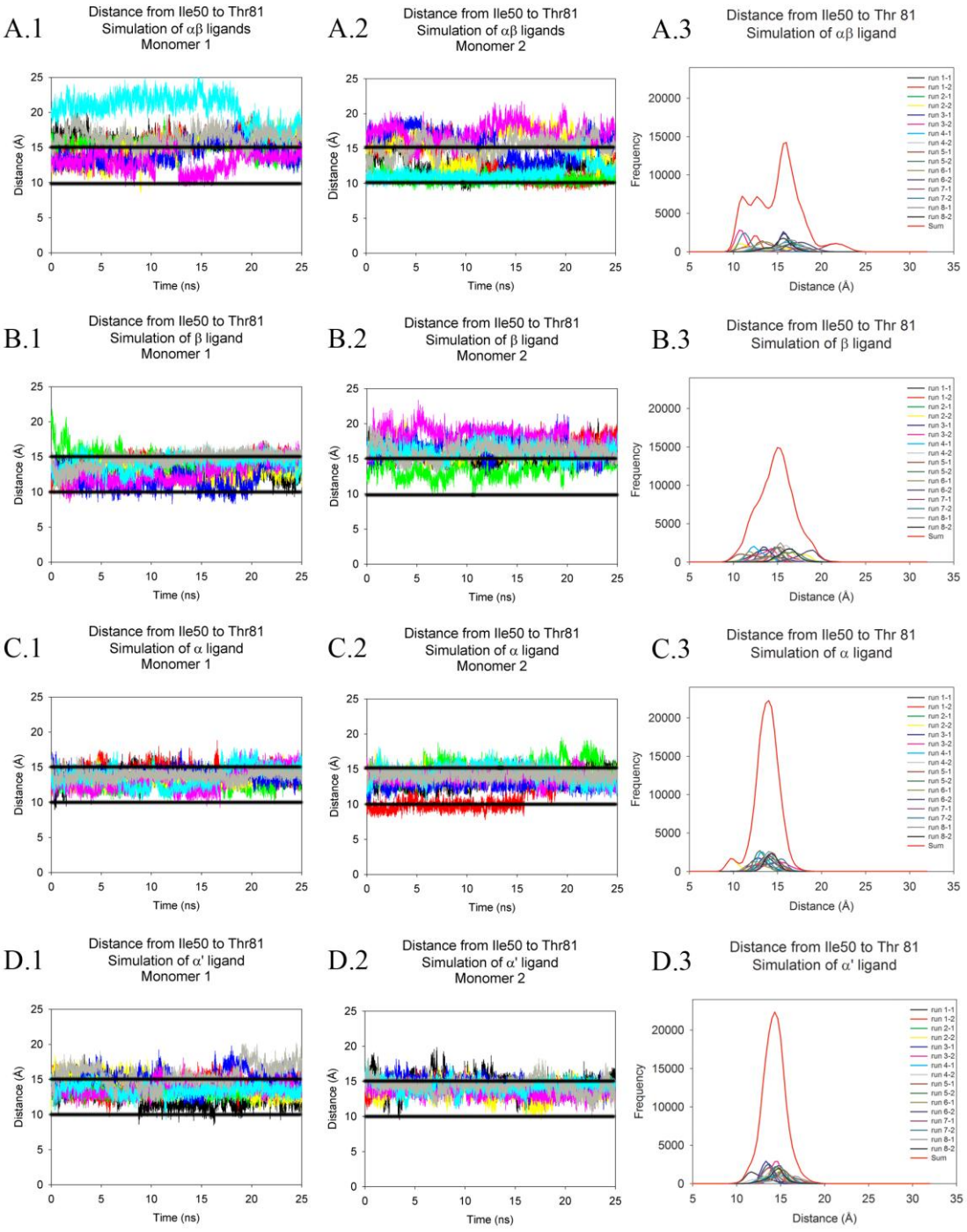

Figure S4: The distance between the flap tip Cα to the 80s loop Cα for monomers 1 and 2 over the length of the production run. The distance fluctuates mainly between 10-20 Å throughout our simulations, but on average is much higher for the trajecories of αβ (A) and β (B), while α (C) and α' (D) occupy a narrower range of distances. The typical closed (15 Å) and semi-open (10 Å) distances are indicated with a black line.

The stability of the bound ligands was judged by several metrics. The simplest stability measure is the root-mean-square deviation of the ligand from its crystallographic position over time. This was calculated for each independent simulation. In addition, the placement of the pyrrole moiety of the ligand within the active site was examined based on the distance between the nitrogen of the pyrrole to the center of mass of the carboxylate carbon of the catalytic aspartic acids.

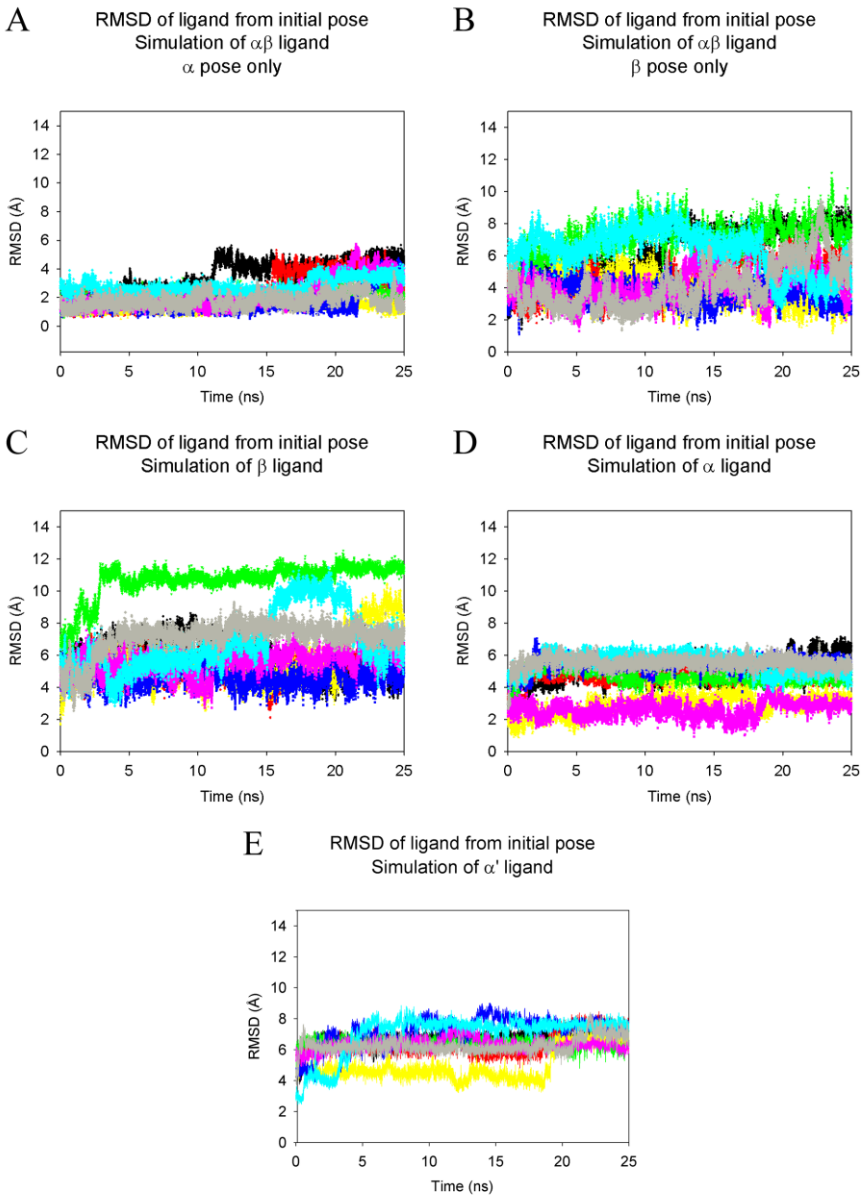

Figure S5: The overall RMSD calculated for each ligand within its protein-ligand complex, yielding the RMSD for α-only in HIVp+αβ (A), β-only in HIVp+αβ (B), β in HIVp+β (C), α in HIVp+α (D), and α' in HIVp+α' (E) over the length of the production run. Trajectories were first fit to the Cα core of the 3BC4 crystal structure.

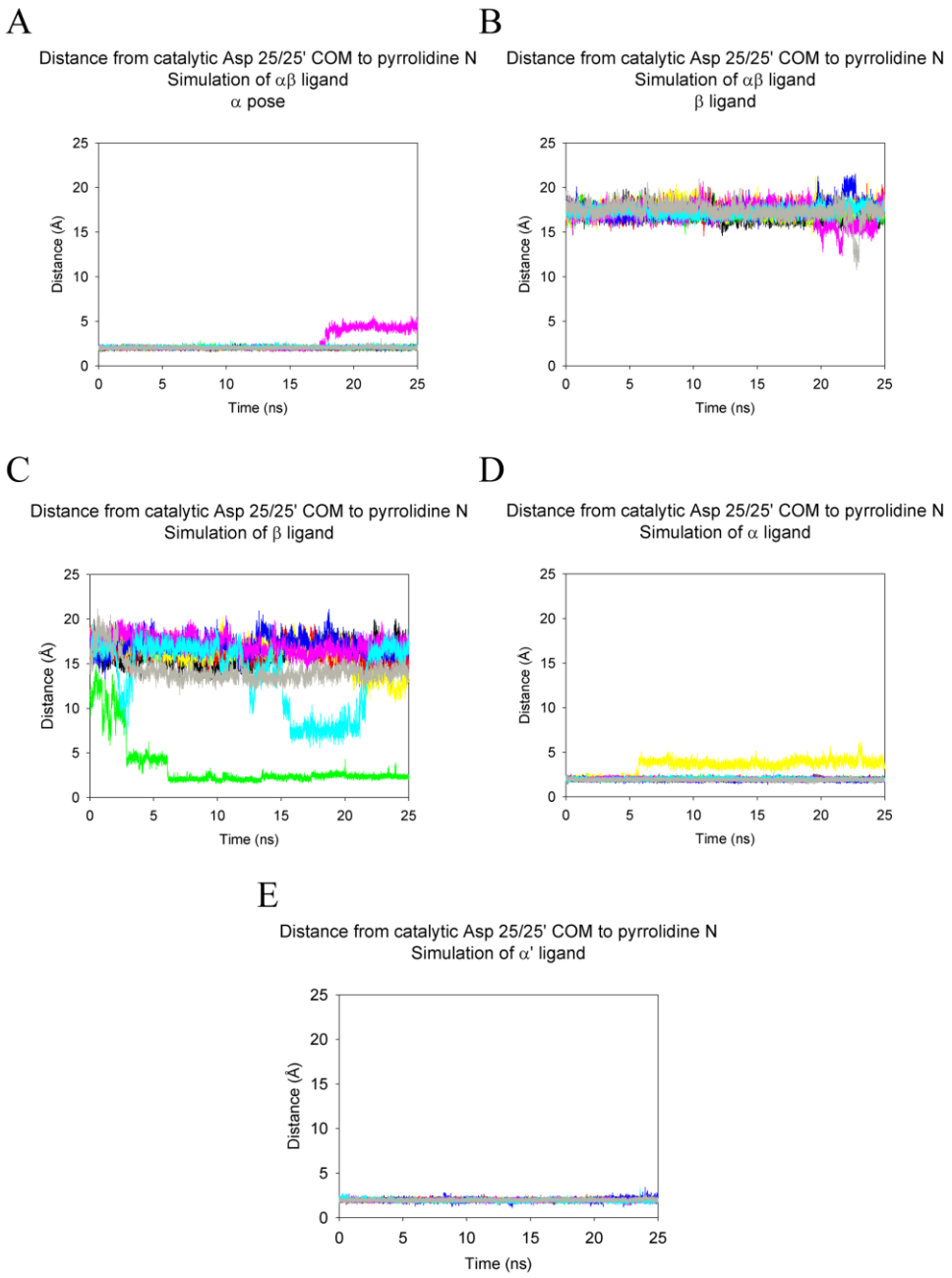

Figure S6: The distance from the center of mass (COM) of the catalytic aspartic acids 25/25’ to the pyrrole nitrogen on the ligand, for α-only in HIVp+αβ (A), β-only in HIVp+αβ (B), β in HIVp+β (C), α in HIVp+α (D), and α’ in HIVp+α’ (E). The green line in C clearly depicts the run in which the ligand flips into the active site (the α position), as well as the run with a partial flip.

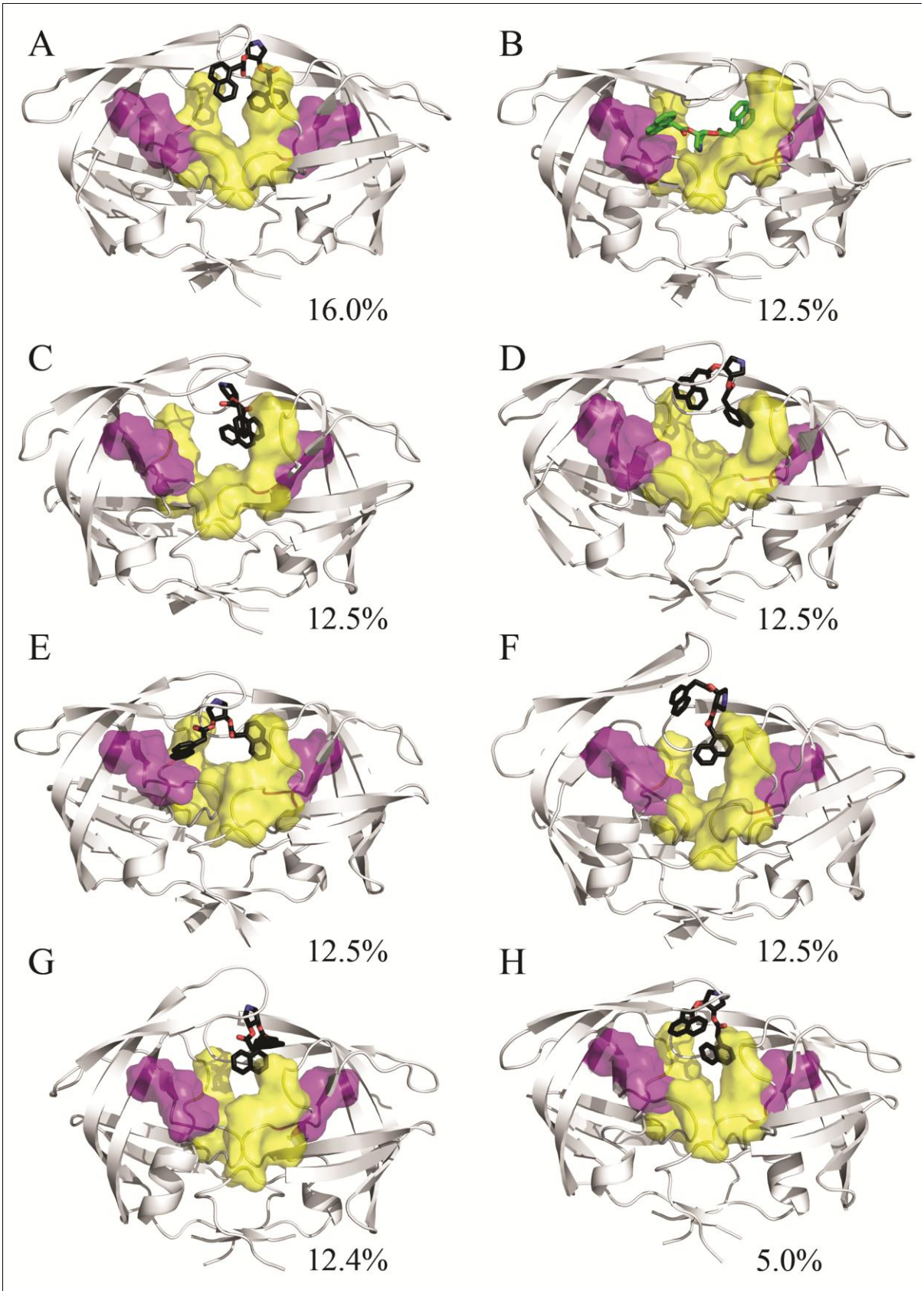

Figure S7: Representative structures from the MD simulation of the HIVp+β complex, taken from the last 5ns of each 25ns trajectory. The β ligand is shown in black, the S1/S1' site is shown in yellow, and the S2/S2' site is shown in purple. The conformational variance of the β ligand is exceptionally high. The β ligand does not preferentially interact with the binding site or eye site, except in the case of (B). This family is the result of one run, where the ligand has moved to bind at the active site, displaying a standard α pose, with contact in one eye site and the S2 pocket.