Supplementary Material: Protein structural memory influences ligand binding mode(s) and unbinding rates

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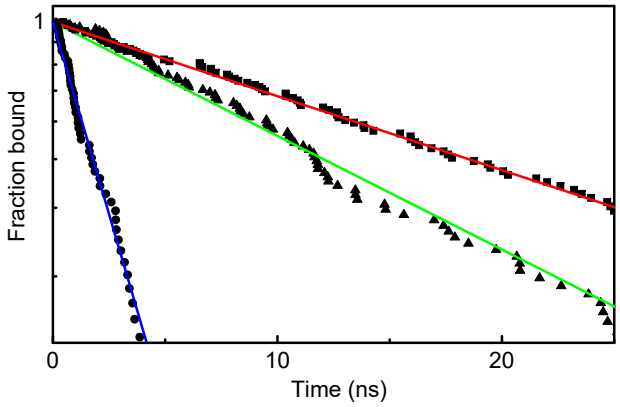

FIG. S1: Fraction of bound DMSO using a threshold of 12 Å. Red and blue lines and corresponding data points are the same as in Fig. 3 of the main text. The triangles and green line originate from unbinding simulations started from the X-ray structure 1D7H (see Methods). The solid lines are exponential fits with unbinding constants $k_{off}^{-1} = 23$ ns for the holo-relaxed protein (red), $k_{off}^{-1} = 15$ ns for the crystal structure (green), and $k_{off}^{-1} = 2.2$ ns for the apo-relaxed protein (blue).

TABLE I: A summary of all simulations performed in this study.

Simulation description	Starting structure	Length of each run	# of independent runs
1. Relaxation, bound state	X-ray	≈ 1.4 μs	2
2. Unbinding	ensemble from 1.	150 ns	116
3. Relaxation without DMSO	end structures of 2.	≈ 500 ns	111
4. Rebinding	end structures of 3.	30 ns	58
5. Unbinding, backbone constrained	X-ray	100 ns	100

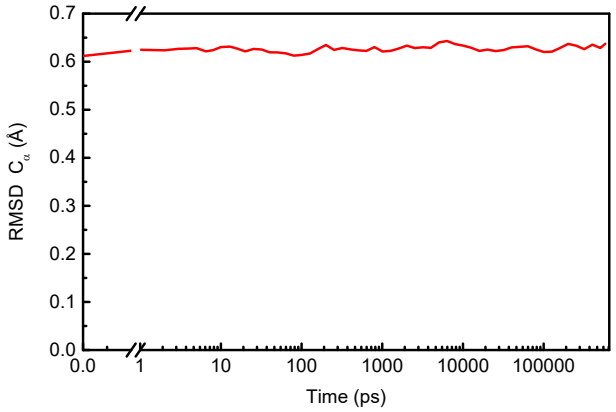

FIG. S2: RMSD of the structures within one time-bin relative to the average structure in the same time-bin. The small RMSD provides evidence that the procedure used for the structural alignment of the MD snapshots for calculating the water density does not introduce artefacts.

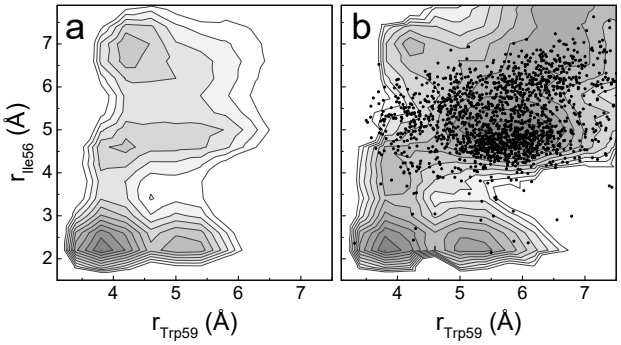

FIG. S3: Same as Fig. 4 in the main text, except for the dots in panel b which correspond to the conformations in which the distance between the DMSO oxygen and the hydroxyl hydrogen atom of Tyr82 is below 2 Å.

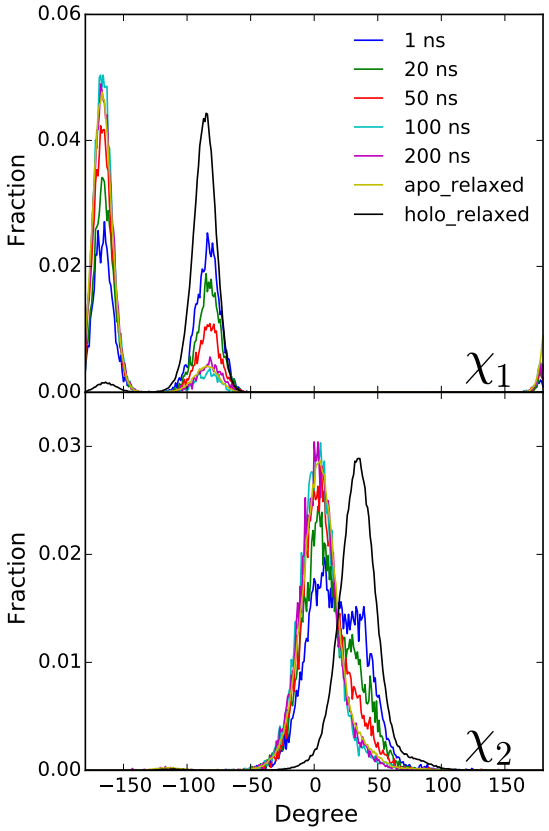

FIG. S4: The distribution of χ_1 and χ_2 dihedral angles of Trp59 shift as a function of time. There are two different conformers of this residue in the apo- and holo-form, respectively. Upon ligand unbinding, there is a progressive shift in the population which converges within 100 ns. The distributions remain nearly constant afterwards.

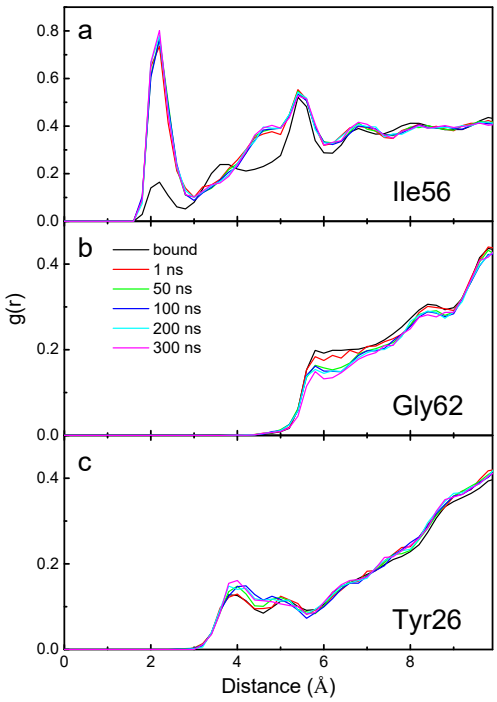

FIG. S5: Radial distribution function $g(r)$ of oxygen atoms of water around the amide proton of Ile56 (a), Gly62 (b), and Tyr26 (c) in the DMSO-bound state (black), as well as 1 ns (red), 50 ns (green), 100 ns (blue), 200 ns (cyan), and 300 ns (magenta) after unbinding from the holo-relaxed protein.

Fig. S5a shows the water structure around the amide proton of Ile56. It is that proton to which DMSO hydrogen-bonds in its most tightly bound configuration (see Fig. 1), and indeed, the corresponding first water peak in the radial distribution function at ≈ 2 Å is small in that case (see Fig. S5a, black line; the peak doesn't vanish completely, since the DMSO molecule also has other non-hydrogen-bonded binding modes). But already 1 ns after unbinding (Fig. S5a, red line), the DMSO molecule is replaced by a hydrogen-bonded water molecule, again as seen from the first peak in the radial distribution function. Water is structured out to ≈ 8 Å, as evidenced by a series of peaks. As a function of time up to 300 ns, the radial distribution function does essentially not evolve any further close to the position of DMSO (Fig. S5a green, blue cyan and magenta lines), while it evolves far away from the binding site (Fig. S5b,c).

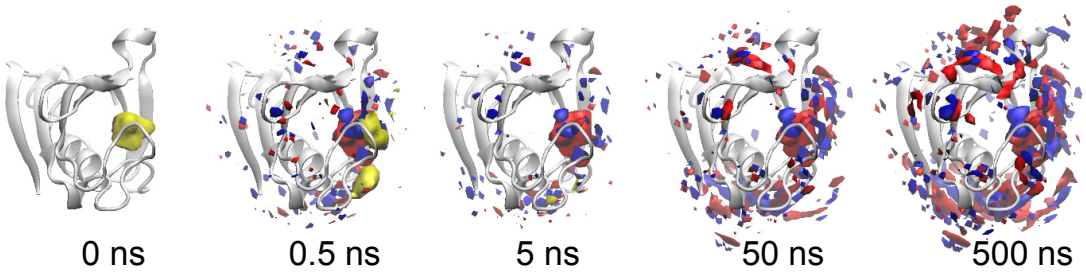

FIG. S6: Change of water density $\Delta\rho$ as a function of time after unbinding from the holo-relaxed protein compared to that just before. Red and blue colors depict increased and decreased water density, respectively. The contour surfaces correspond to changes of ± 0.013 waters/Å^3 (for comparison, the bulk water density is ≈ 0.033 waters/Å^3). The protein is shown by a gray ribbon, and the DMSO density in yellow.

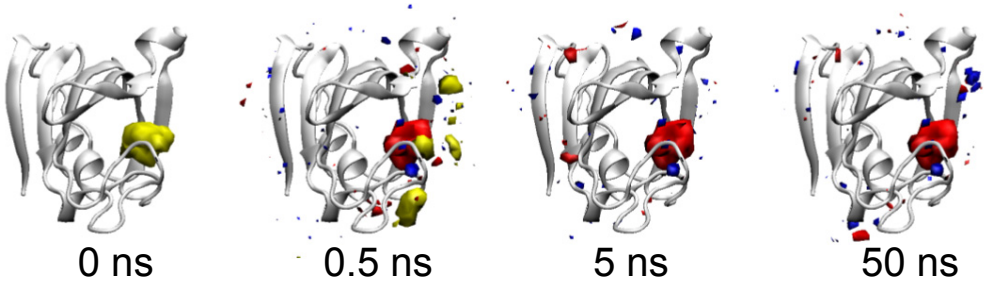

FIG. S7: Same as Fig. S6, but keeping the protein backbone rigid during the unbinding simulations. In this case, a simpler and computationally less expensive protocol has been used to collect an ensemble of starting structures. That is, the DMSO molecule was forced to stay in the binding pocket by center-of-mass pulling with a harmonic spring with a force constant of 1000 kJ/mol·nm^2. The system was then allowed to equilibrate for 100 ns with all other degrees of freedoms free, and 100 snapshots were taken from a subsequent 10 ns run. For the production runs (each of which was 100 ns long), the protein backbone atoms was constrained to the position of the corresponding starting structure with a force constant of 1000 kJ/mol·nm^2. All other procedures were the same as described in Methods.