Electronic Supplementary Material (ESI) for Faraday Discussions.

Harnessing Conformational Dynamics in Enzyme Catalysis to achieve Nature-like catalytic efficiencies: The Shortest Path Map tool for computational enzyme design

Cristina Duran,[a] Guillem Casadevall [a] and Sílvia Osuna *[a,b]

Supporting Information

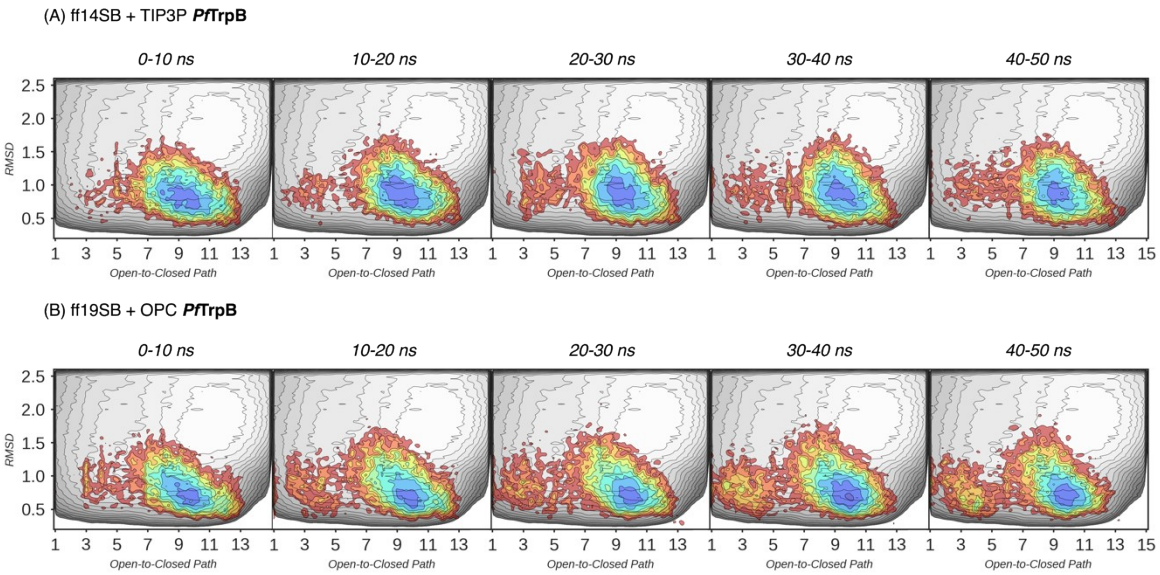

Figure S1. Free Energy Landscapes (FEL) of *Pf*TrpB estimated each additional 10 ns of MD simulation time. The estimated FEL from the 10 ns accumulated multiple replica short nanosecond timescale MD simulations performed starting at the ensemble of template-based AF2 predictions is shown in color on top of the previously reconstructed FEL of the *Pf*TrpB variant (shown in gray scale).[1] FELs are computed every 10 ns of MD simulation time: 0-10 ns, 20-30 ns, 30-40 ns, and 40-50 ns. Two different combinations of forcefield and water model: (A) ff14SB and TIP3P water, and (B) ff19SB and OPC water. The x axis denotes the open-to-closed transition of the COMM domain, which ranges from 1-5 (open, **O**), 6-10 (partially closed, **PC**), to 11-15 (closed, **C**), the y axis is the mean square deviation (MSD) deviation from the path of **O**-to-**C** structures generated. Most stable conformations are shown in blue, whereas higher in energy regions in red.

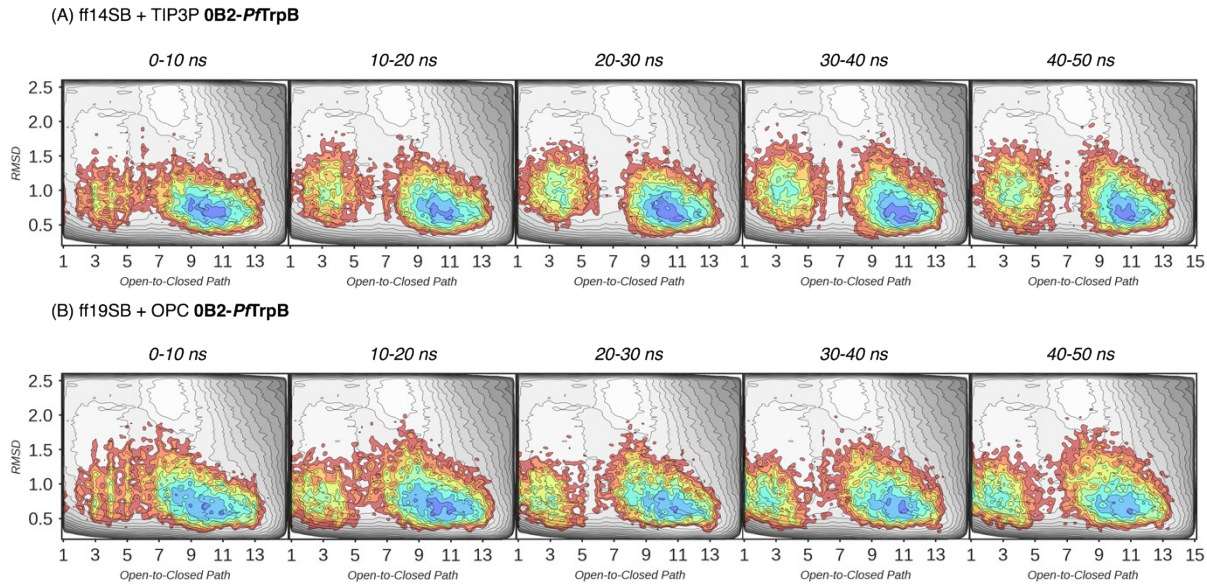

Figure S2. Free Energy Landscapes (FEL) of 0B2-*Pf*TrpB estimated each additional 10 ns of MD simulation time. The estimated FEL from the 10 ns accumulated multiple replica short nanosecond timescale MD simulations performed starting at the ensemble of template-based AF2 predictions is shown in color on top of the previously reconstructed FEL of the *Pf*TrpB variant (shown in gray scale).[1] FELs are computed every 10 ns of MD simulation time: 0-10 ns, 20-30 ns, 30-40 ns, and 40-50 ns. Two different combinations of forcefield and water model: (A) ff14SB and TIP3P water, and (B) ff19SB and OPC water. The x axis denotes the open-to-closed transition of the COMM domain, which ranges from 1-5 (open, **O**), 6-10 (partially closed, **PC**), to 11-15 (closed, **C**), the y axis is the mean square deviation (MSD) deviation from the path of **O**-to-**C** structures generated. Most stable conformations are shown in blue, whereas higher in energy regions in red.

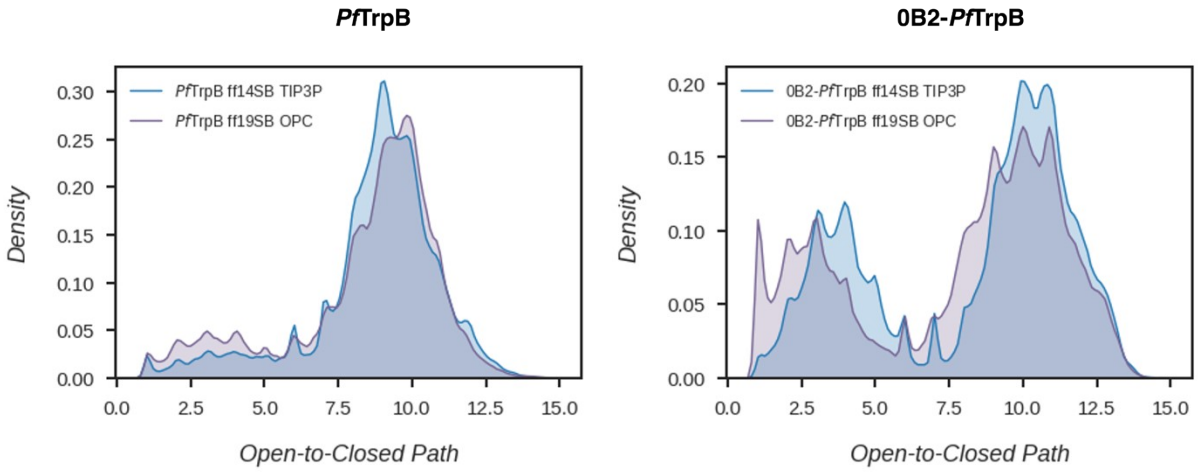

Figure S3. Histogram of the open and closed conformations of the COMM domain in sampled in the MD simulation data *Pf*TrpB and 0B2-*Pf*TrpB. Different colors have been used for the two different combinations of forcefield and water model: ff14SB and TIP3P water (in blue), and ff19SB and OPC water (purple).

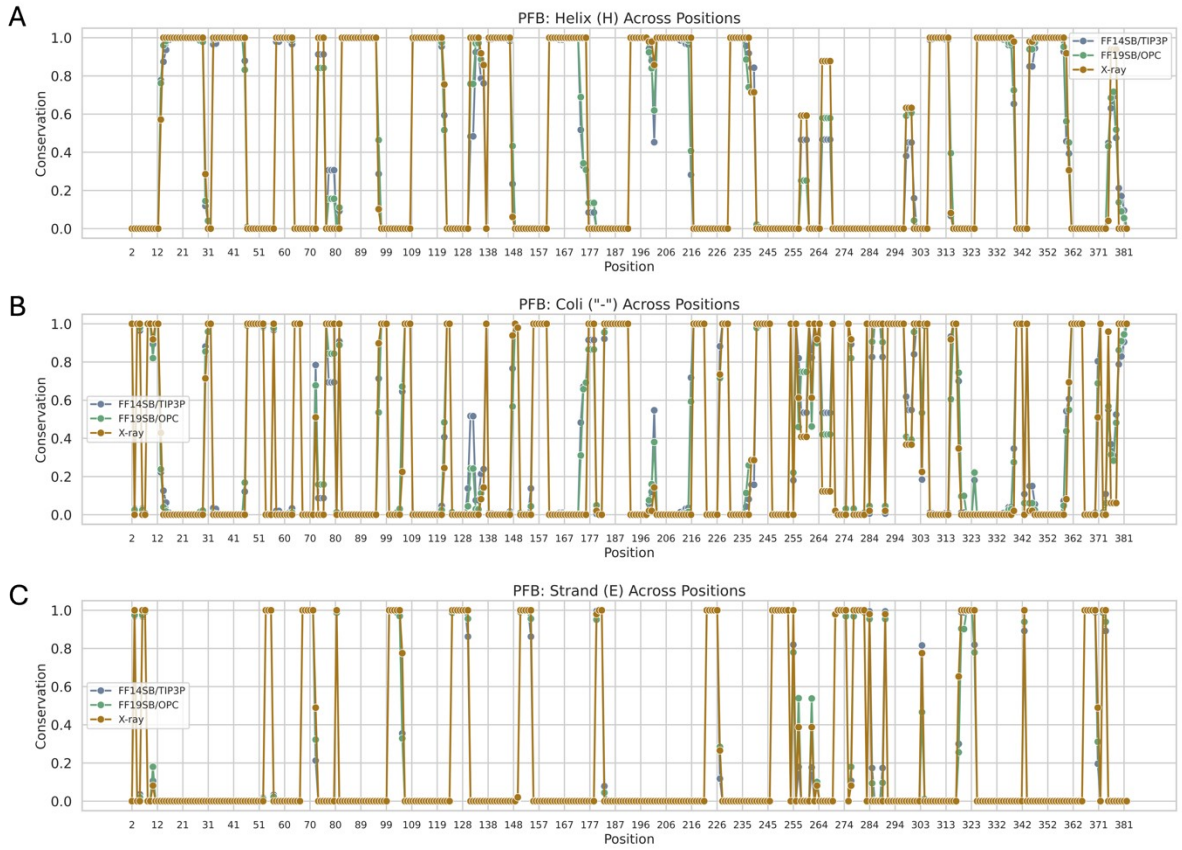

Figure S4. Evaluation of the secondary structure of *Pf*TrpB: (A) alpha helices, (B) loops, and (C) beta strand according to X-ray data (brown line and dots), ff19SB/OPC (blue dots and lines), and ff14SB/TIP3P (green dots and lines). The following X-rays were considered: 6AMH_A, 6AMH_C, 6AMH_D, 6AMC_A, 6AMC_C, 5VM5_A, 5VM5_C, 6AM8_A, 6AM8_B, 6AM8_C, 6AM9_A, 6AM9_C, 6AM9_D, 6AM7_A, 6AM7_C, 5IXJ_A, 5IXJ_C, 5E0K_B, 5E0K_D, 5E0K_F, 5E0K_H, 5E0K_J, 5E0K_L, 5DW0_A, 5DW0_B, 5DW0_C, 5DW0_D, 1V8Z_A, 1V8Z_B, 1V8Z_C, 1V8Z_D, 5T6M_B, 5T6M_C, 1WDW_B, 1WDW_D, 1WDW_F, 1WDW_H, 1WDW_J, 1WDW_L, 5DVZ_A, 5DVZ_D, 6CUV_A, 6CUV_C, 6CUV_D, 6CUT_A, 6CUT_B, 6CUT_D, 6CUZ_A,

6CUZ_D. The algorithm used for assigning the secondary structure is PyDSSP (https://github.com/ShintaroMinami/PyDSSP).[2]

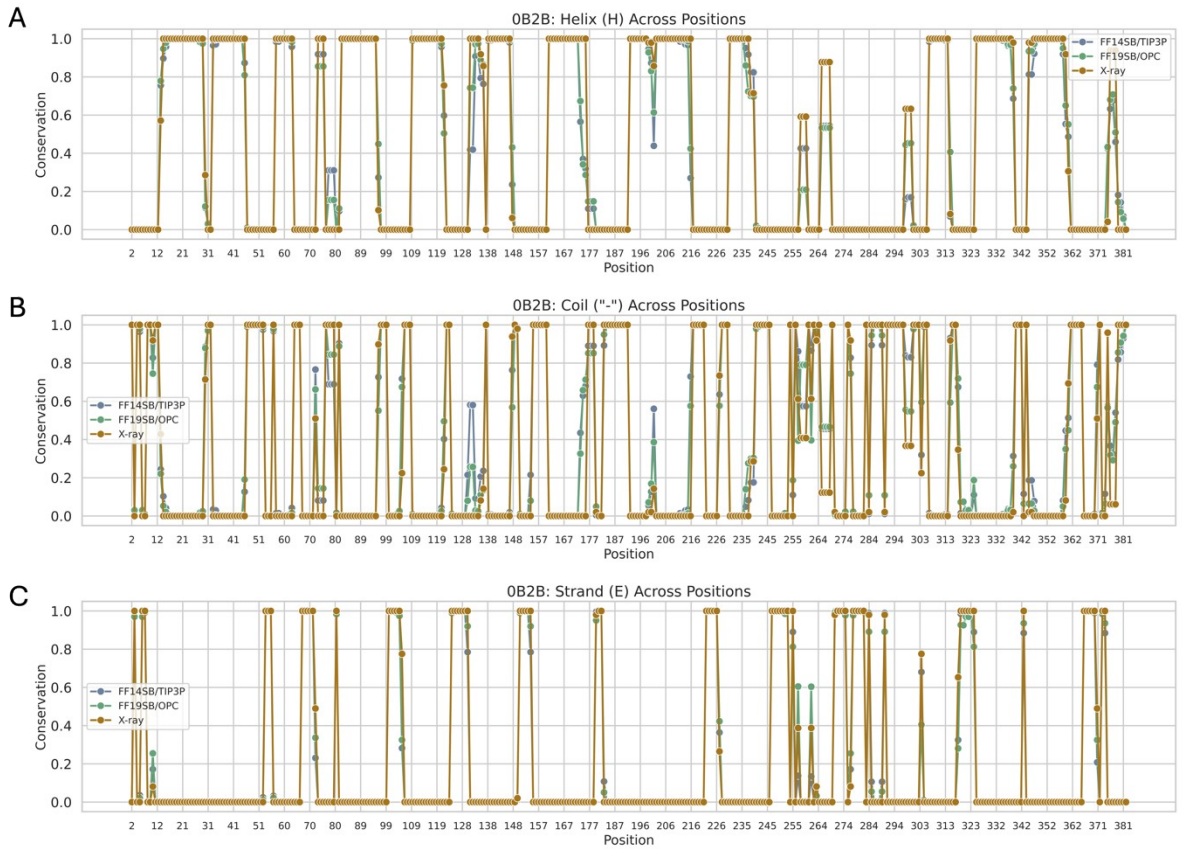

Figure S5. Evaluation of the secondary structure of 0B2-*Pf*TrpB: (A) alpha helices, (B) loops, and (C) beta strand according to X-ray data (brown line and dots), ff19SB/OPC (blue dots and lines), and ff14SB/TIP3P (green dots and lines). The following X-rays were considered: 6AMH_A, 6AMH_C, 6AMH_D, 6AMC_A, 6AMC_C, 5VM5_A, 5VM5_C, 6AM8_A, 6AM8_B, 6AM8_C, 6AM9_A, 6AM9_C, 6AM9_D, 6AM7_A, 6AM7_C, 5IXJ_A, 5IXJ_C, 5E0K_B, 5E0K_D, 5E0K_F, 5E0K_H, 5E0K_J, 5E0K_L, 5DW0_A, 5DW0_B, 5DW0_C, 5DW0_D, 1V8Z_A, 1V8Z_B, 1V8Z_C, 1V8Z_D, 5T6M_B, 5T6M_C, 1WDW_B, 1WDW_D, 1WDW_F, 1WDW_H, 1WDW_J, 1WDW_L, 5DVZ_A, 5DVZ_D, 6CUV_A, 6CUV_C, 6CUV_D, 6CUT_A, 6CUT_B, 6CUT_D, 6CUZ_A, 6CUZ_D. The algorithm used for assigning the secondary structure is PyDSSP (https://github.com/ShintaroMinami/PyDSSP).[2]

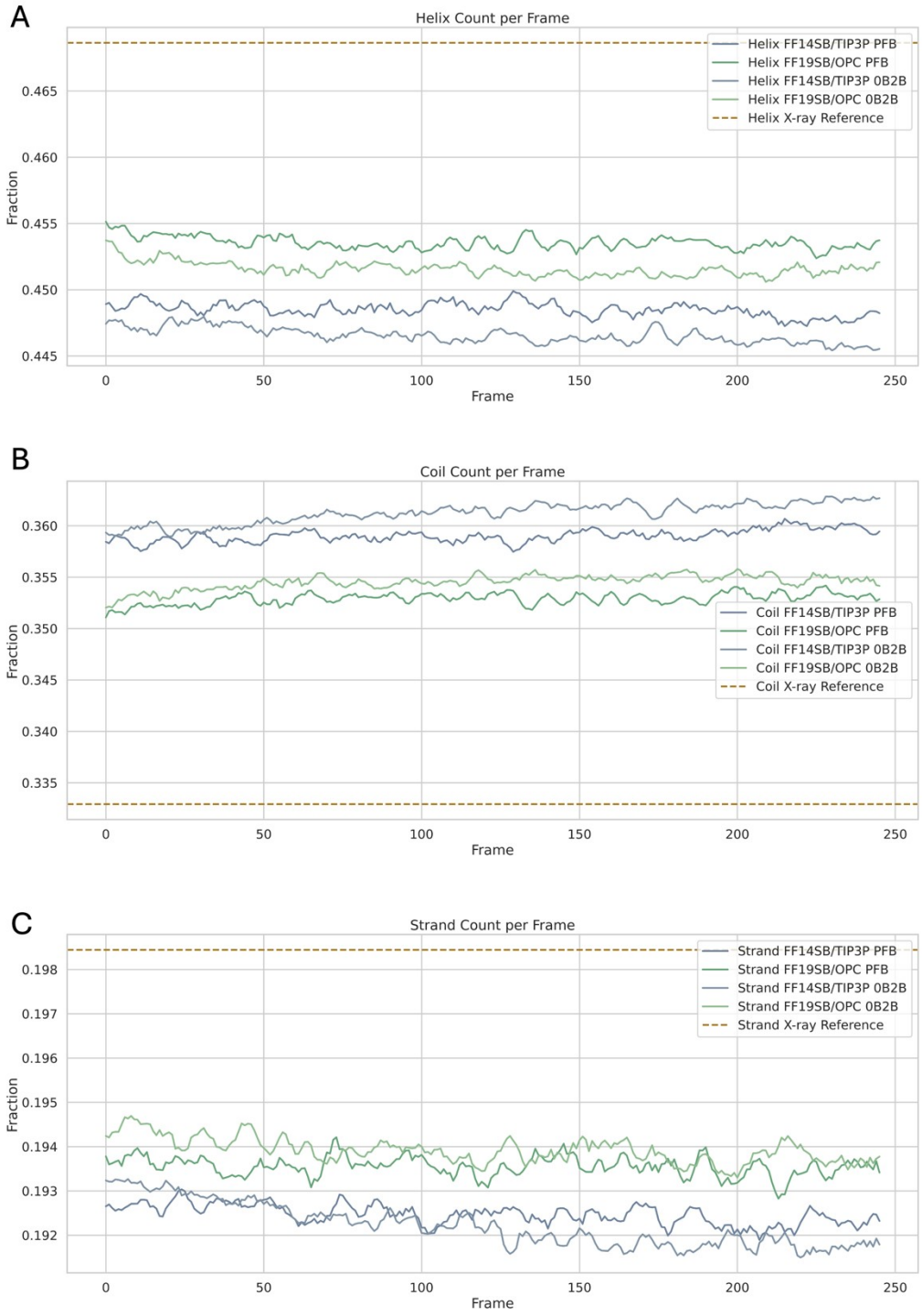

Figure S6. Evaluation of the secondary structure along the MD simulation frames for: (A) alpha helices, (B) loops, and (C) beta strand using either ff19SB/OPC (dark blue line for *Pf*TrpB, lighter blue for 0B2-*Pf*TrpB) or ff14SB/TIP3P (dark green line for *Pf*TrpB, lighter green for 0B2-*Pf*TrpB). The obtained values for X-ray data are shown with a discontinuous brown line. The following X-rays were considered: 6AMH_A, 6AMH_C, 6AMH_D, 6AMC_A, 6AMC_C, 5VM5_A, 5VM5_C, 6AM8_A, 6AM8_B, 6AM8_C, 6AM9_A, 6AM9_C, 6AM9_D, 6AM7_A, 6AM7_C, 5IXJ_A, 5IXJ_C, 5E0K_B, 5E0K_D, 5E0K_F, 5E0K_H, 5E0K_J, 5E0K_L, 5DW0_A, 5DW0_B, 5DW0_C, 5DW0_D, 1V8Z_A, 1V8Z_B, 1V8Z_C, 1V8Z_D, 5T6M_B, 5T6M_C, 1WDW_B, 1WDW_D, 1WDW_F, 1WDW_H, 1WDW_J, 1WDW_L, 5DVZ_A, 5DVZ_D, 6CUV_A, 6CUV_C, 6CUV_D, 6CUT_A, 6CUT_B, 6CUT_D, 6CUZ_A, 6CUZ_D. The algorithm used for assigning the secondary structure is PyDSSP (https://github.com/ShintaroMinami/PyDSSP).[2] The standard deviations are not shown for visualization purposes, but are in line with the mean trends.

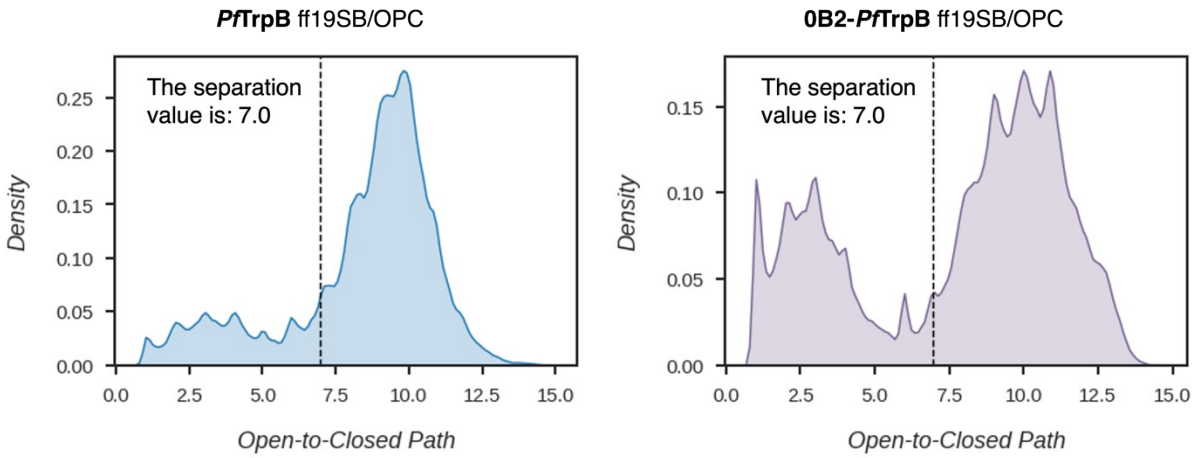

Figure S7. Histogram of the open and closed conformations of the COMM domain in sampled in the last 20 ns of the MD simulation data *Pf*TrpB (in blue) and 0B2-*Pf*TrpB (in purple). All conformations in the (0-7.0) range are considered to have open structures of the COMM domain, and closed structures correspond to the (7.0-15.0).

References:

1. M. A. Maria-Solano, J. Iglesias-Fernández and S. Osuna, *J. Am. Chem. Soc.*, 2019, **141**, 13049-13056.
2. W. Kabsch and C. Sander, *Biopolymers*, 1983, **22**, 2577-2637.