Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Molecular dynamics derived life times of active substrate binding poses explain K_m of laccase mutants

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Table S1. Preparation of the protein-ligand complexes for MD simulation.

Mutation	**Simulation cell size (Å^3)**	**Neutralizing counter ions**	**Total number of atoms in the system**	**Number of water molecules**
WT (pH 3.4)	487813	$8Na^+$	44990	12509
D206A	487819	$7Na^+$	45020	12520
D206N	487799	$7Na^+$	45030	12522
WT (pH 5.0)	487840	$12Na^+$	44936	12491
F332A	487936	$12Na^+$	44935	12494
F337A	487998	$12Na^+$	44932	12493

Table S2. Analysis of the binding energies of 2,6-DMP complexes with six laccase variants.

Mutation	**pH**	**K_m (μM)**	**k_{cat}/K_m (s^{-1} mM)**	**MMGBSA bind affinity at 0 Å flexibility (kcal/mol)**
WT	3.4	190	2.7	-32.7
D206A	3.4	1630	0.4	-30.8
D206N	3.4	3280	0.09	-23.5
WT	5.0	17±5	N/A	-29.6
F332A	5.0	165±5	N/A	-26.4
F337A	5.0	N/A	N/A	-28.1

Supplementary Figures

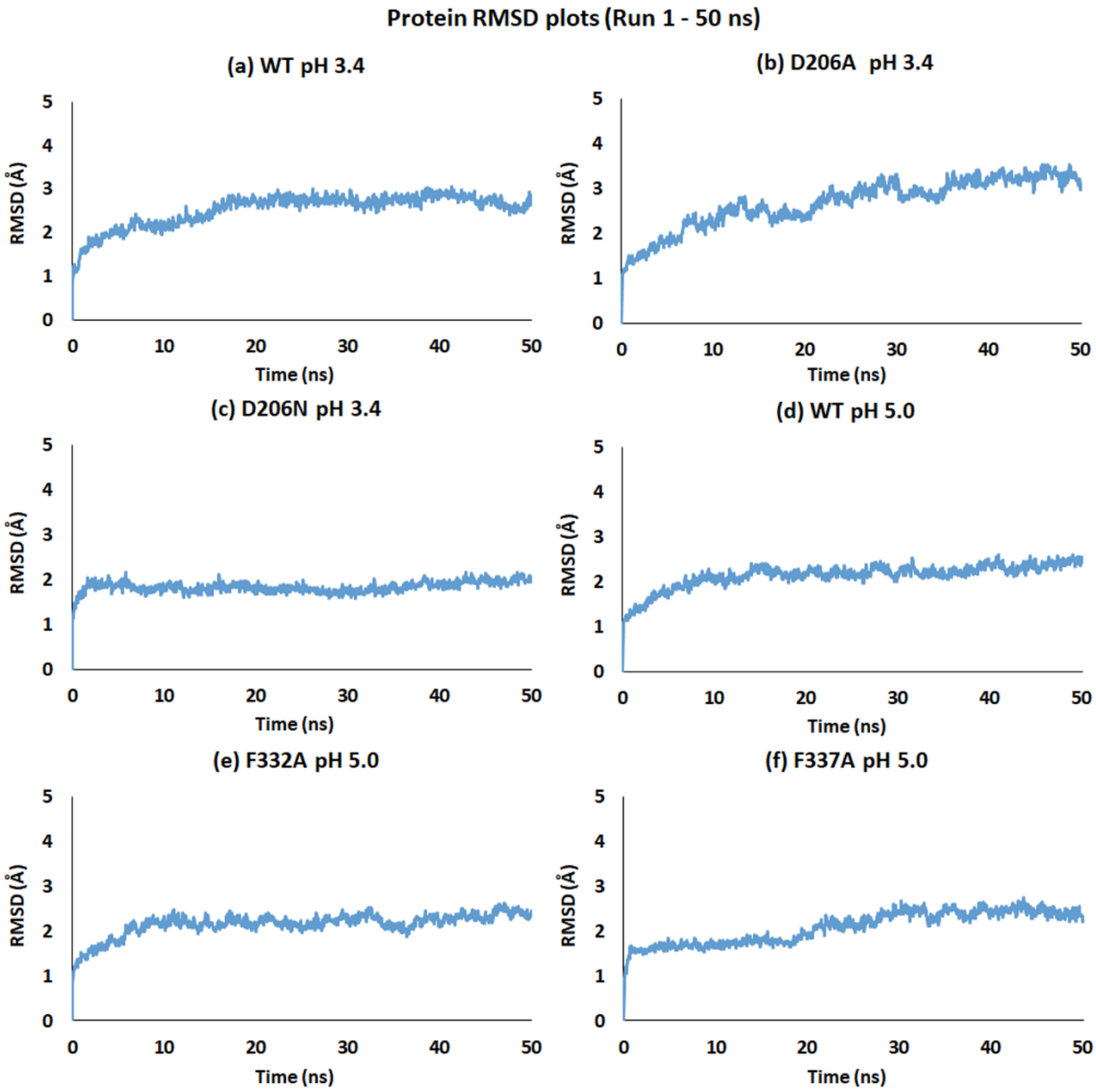


Figure S1. RMSD plots of laccase variants during run 1 (50 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

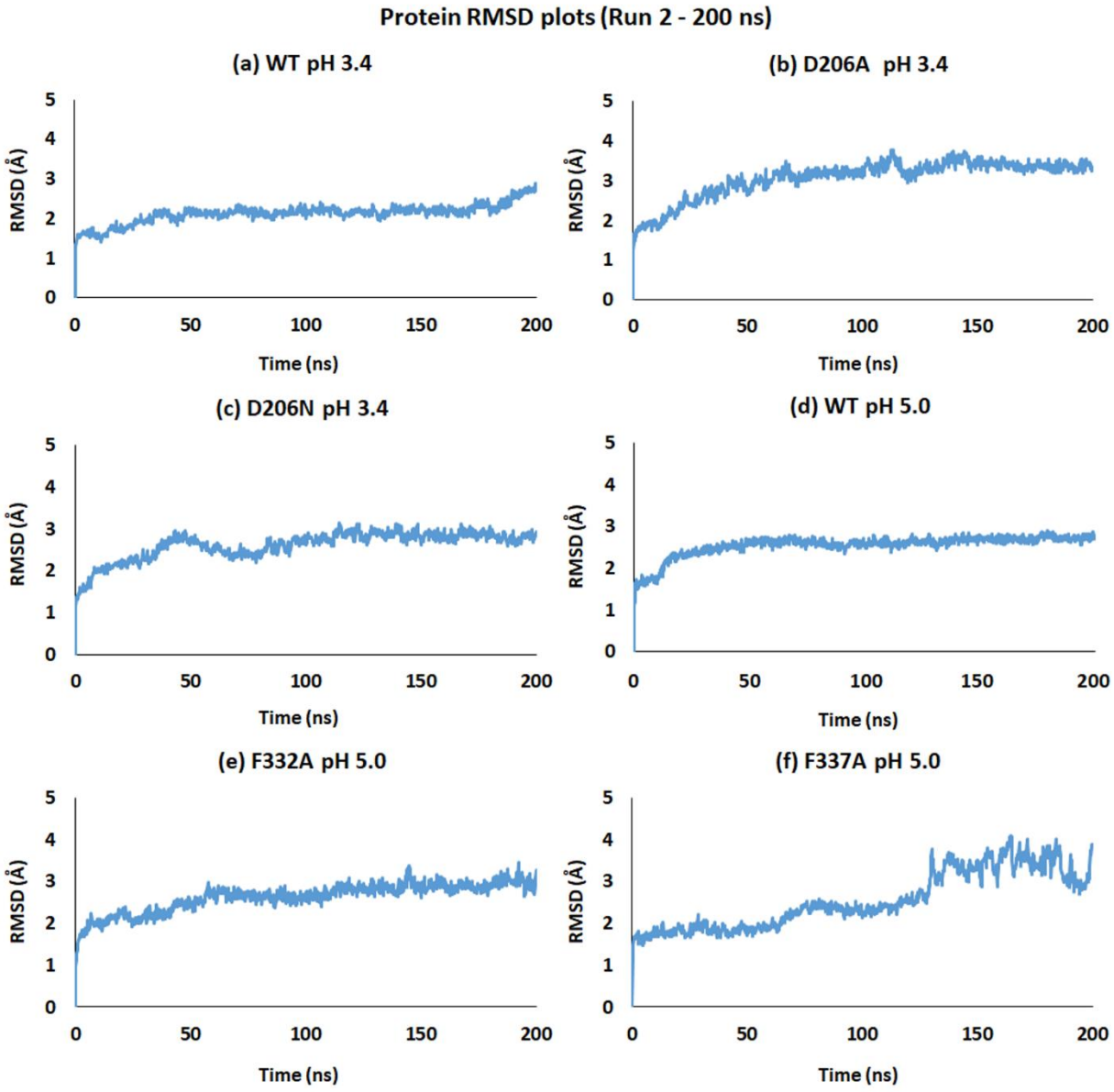


Figure S2. RMSD plots of laccase variants during run 2 (200 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

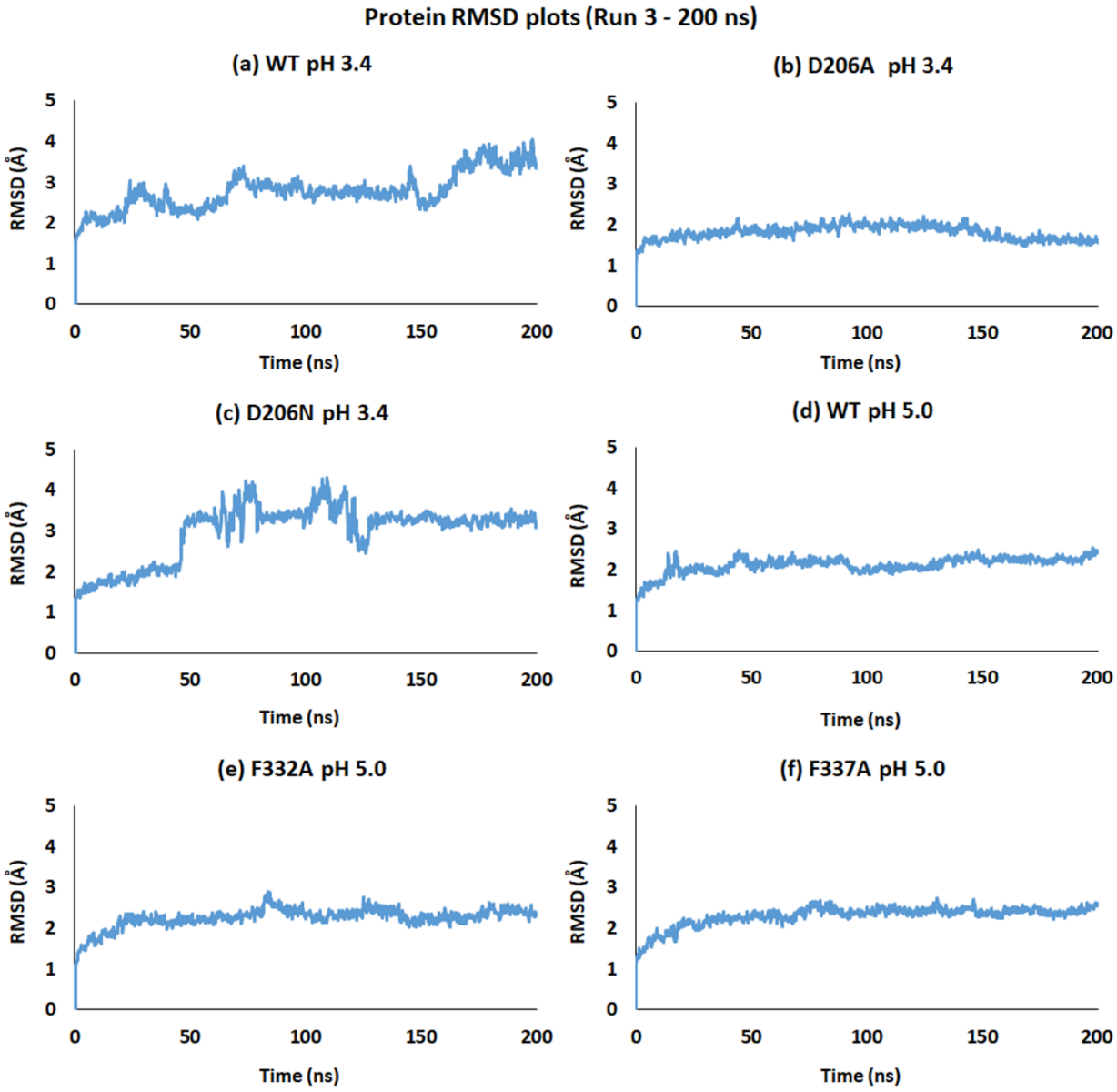


Figure S3. RMSD plots of laccase variants during run 3 (200 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

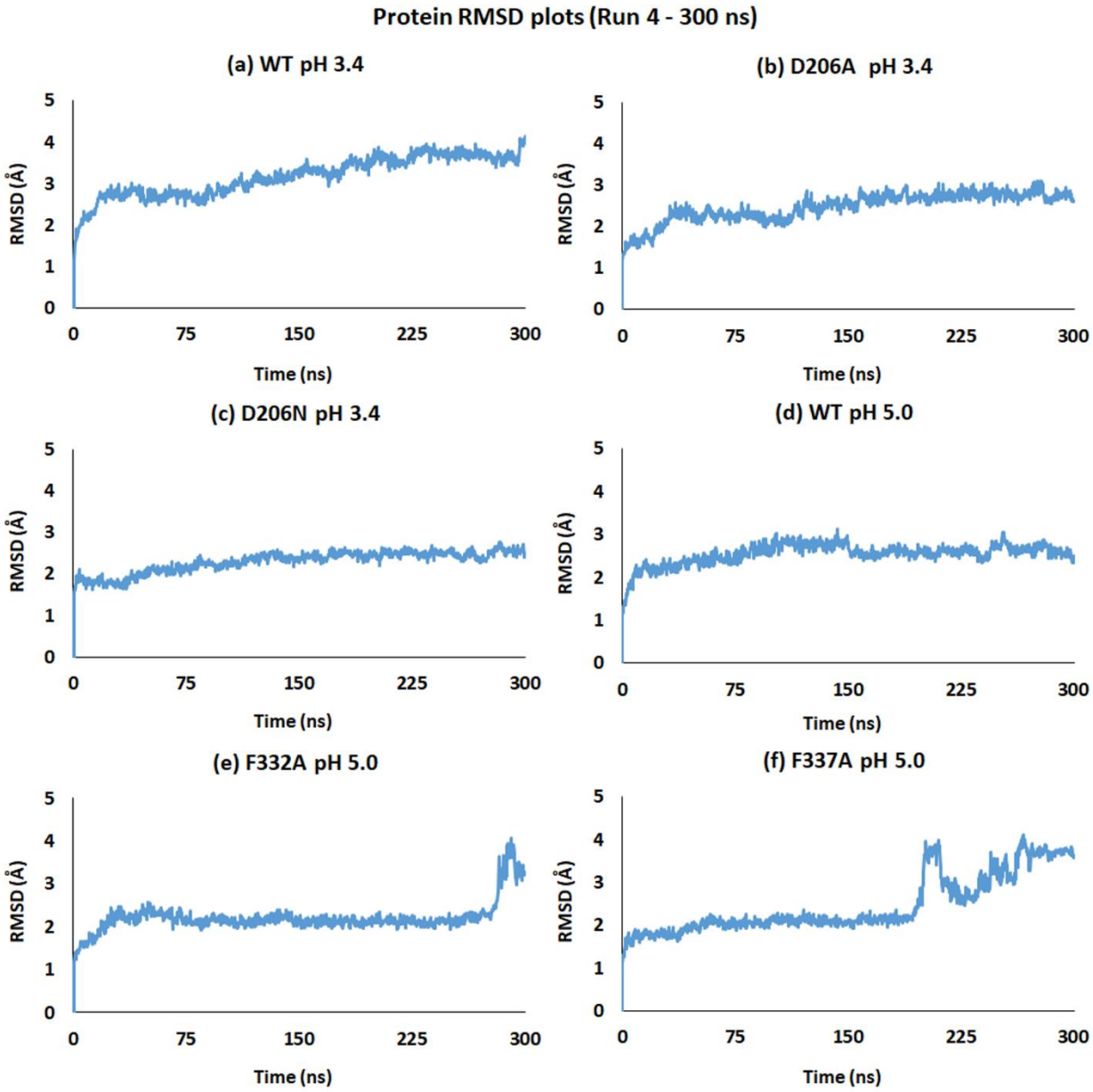


Figure S4. RMSD plots of laccase variants during run 4 (300 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

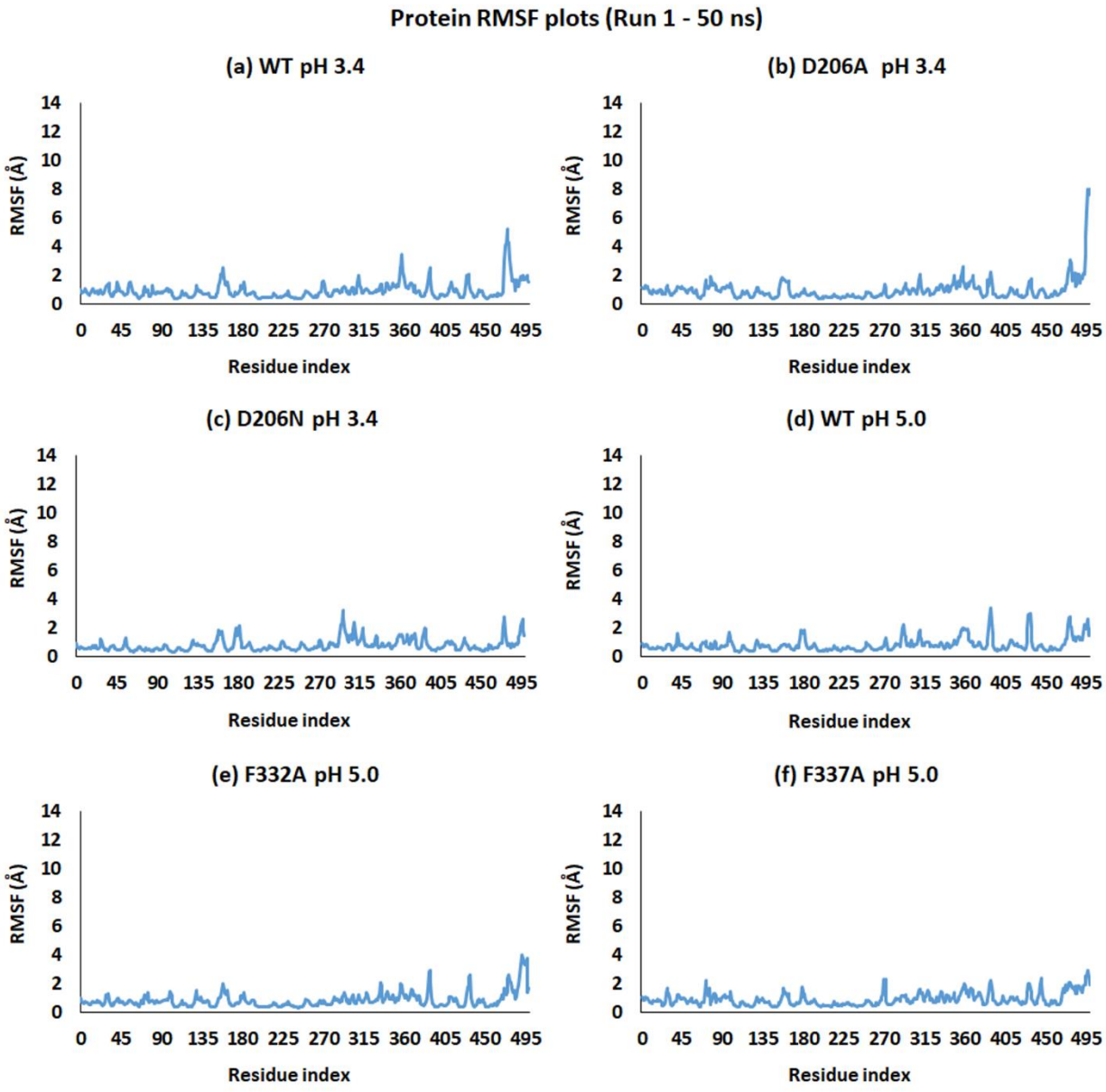


Figure S5. RMSF plots of laccase variants during run 1 (50 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

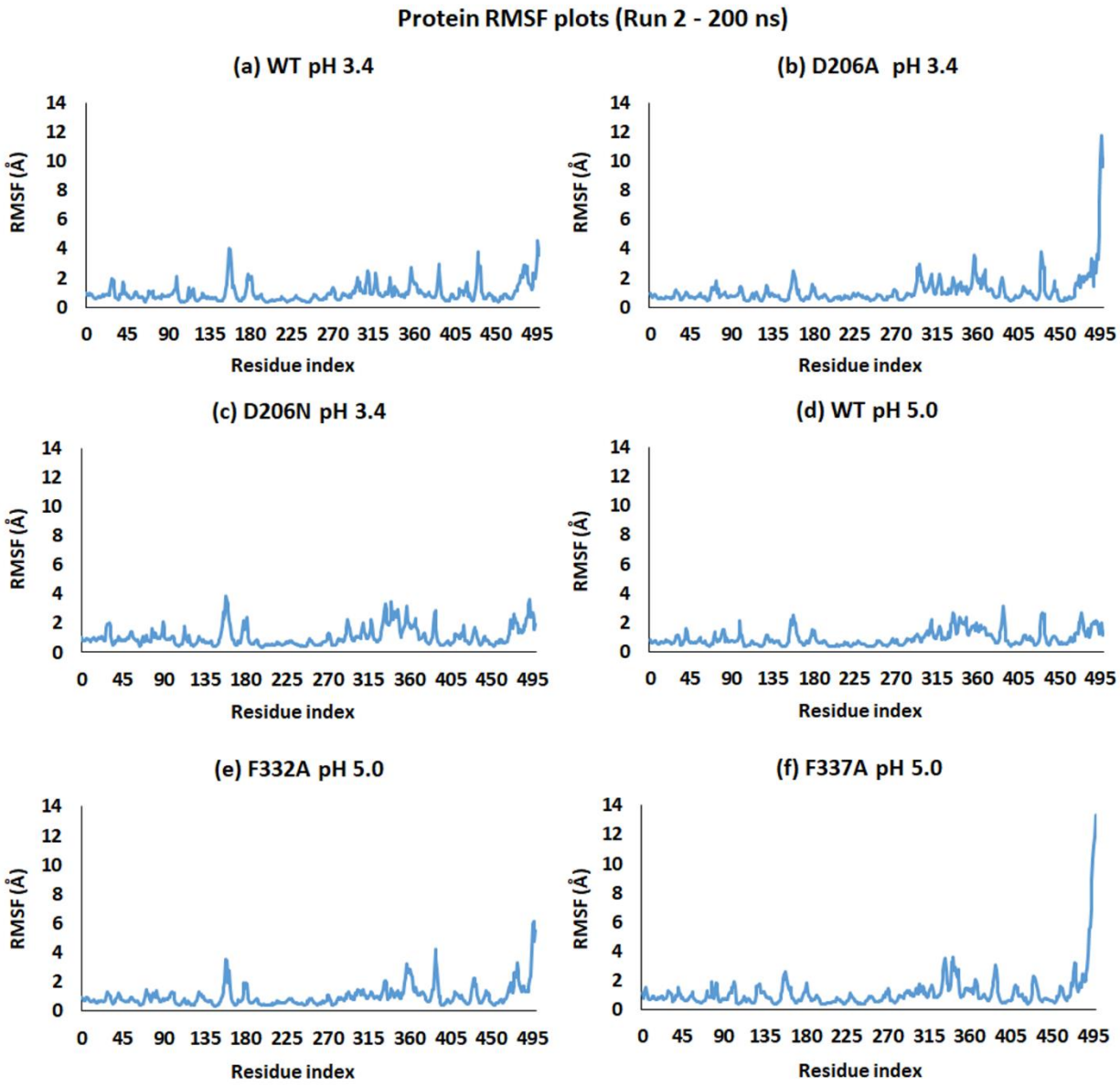


Figure S6. RMSF plots of laccase variants during run 2 (200 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

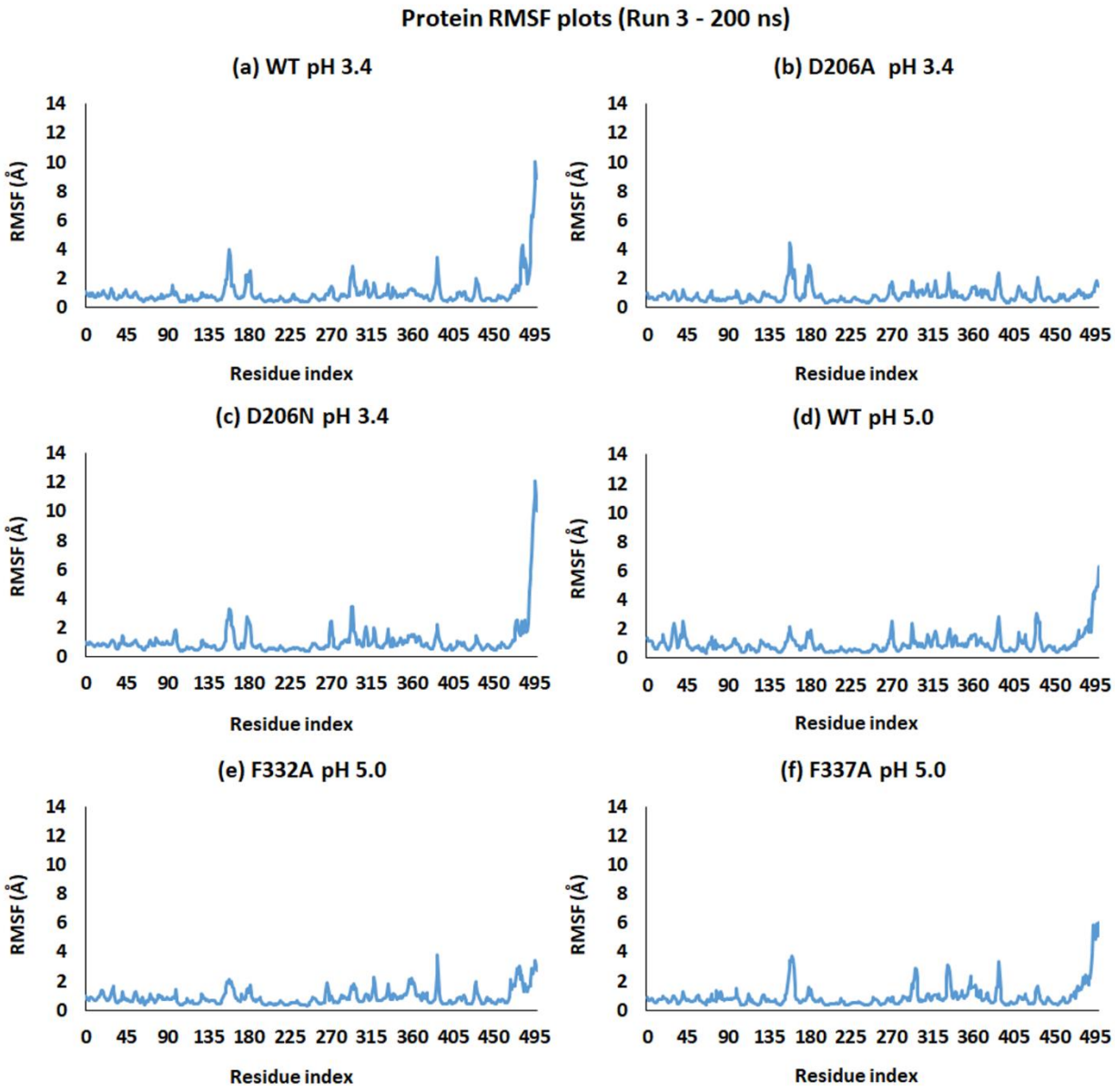


Figure S7. RMSF plots of laccase variants during run 3 (200 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

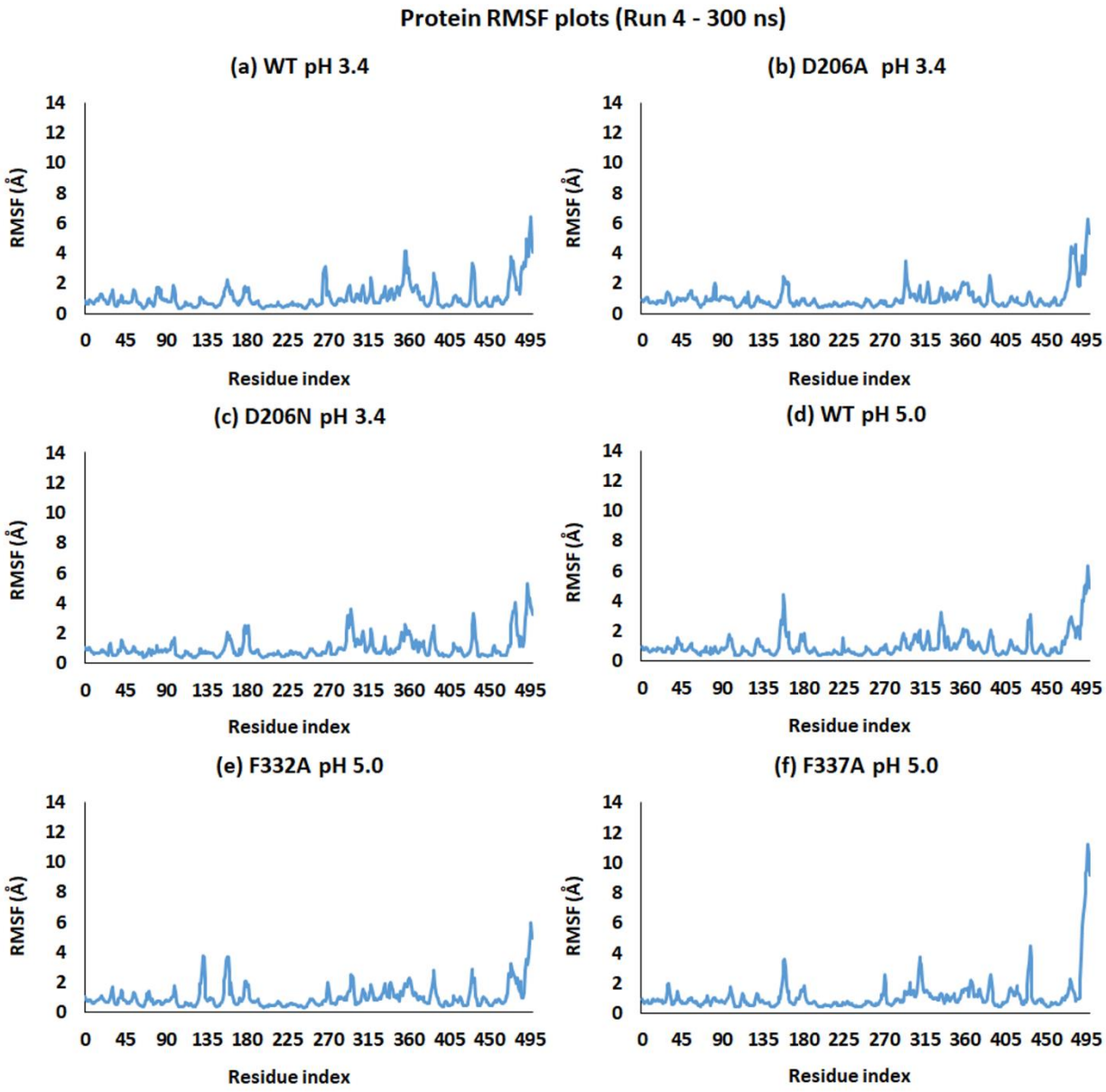


Figure S8. RMSF plots of laccase variants during run 4 (300 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

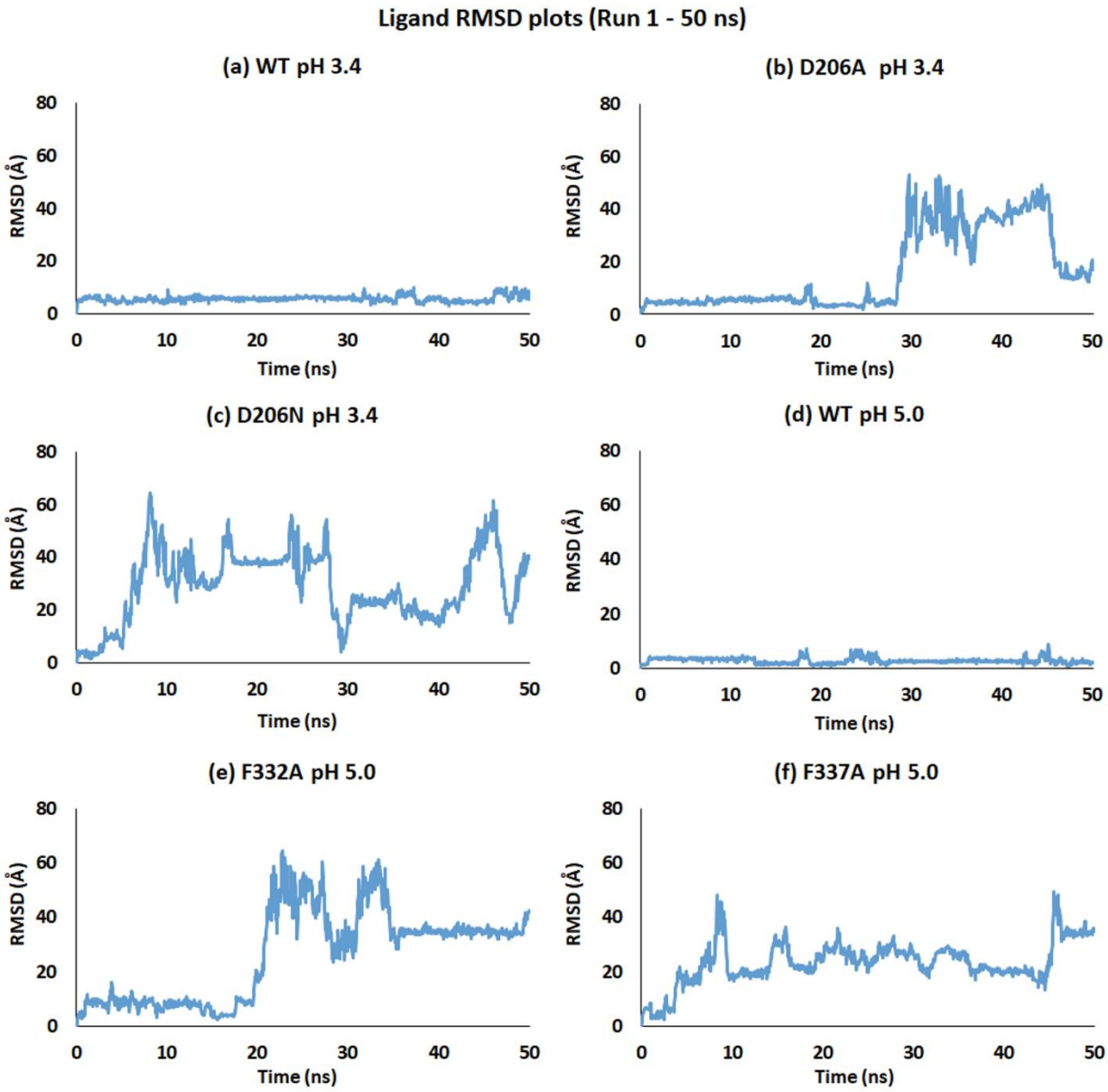


Figure S9. RMSD plots of 2,6-DMP during run 1 (50 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

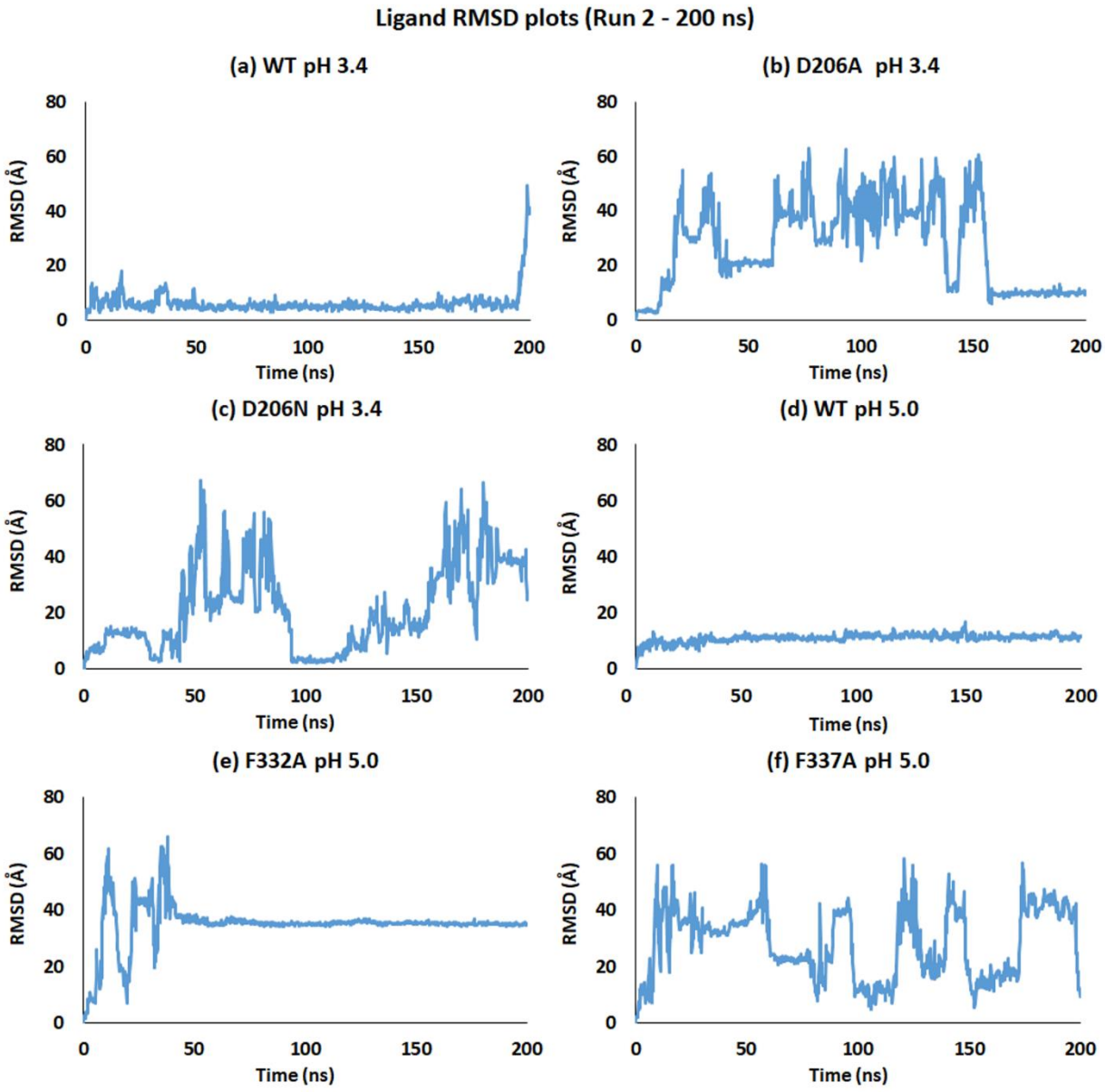


Figure S10. RMSD plots of 2,6-DMP during run 2 (200 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

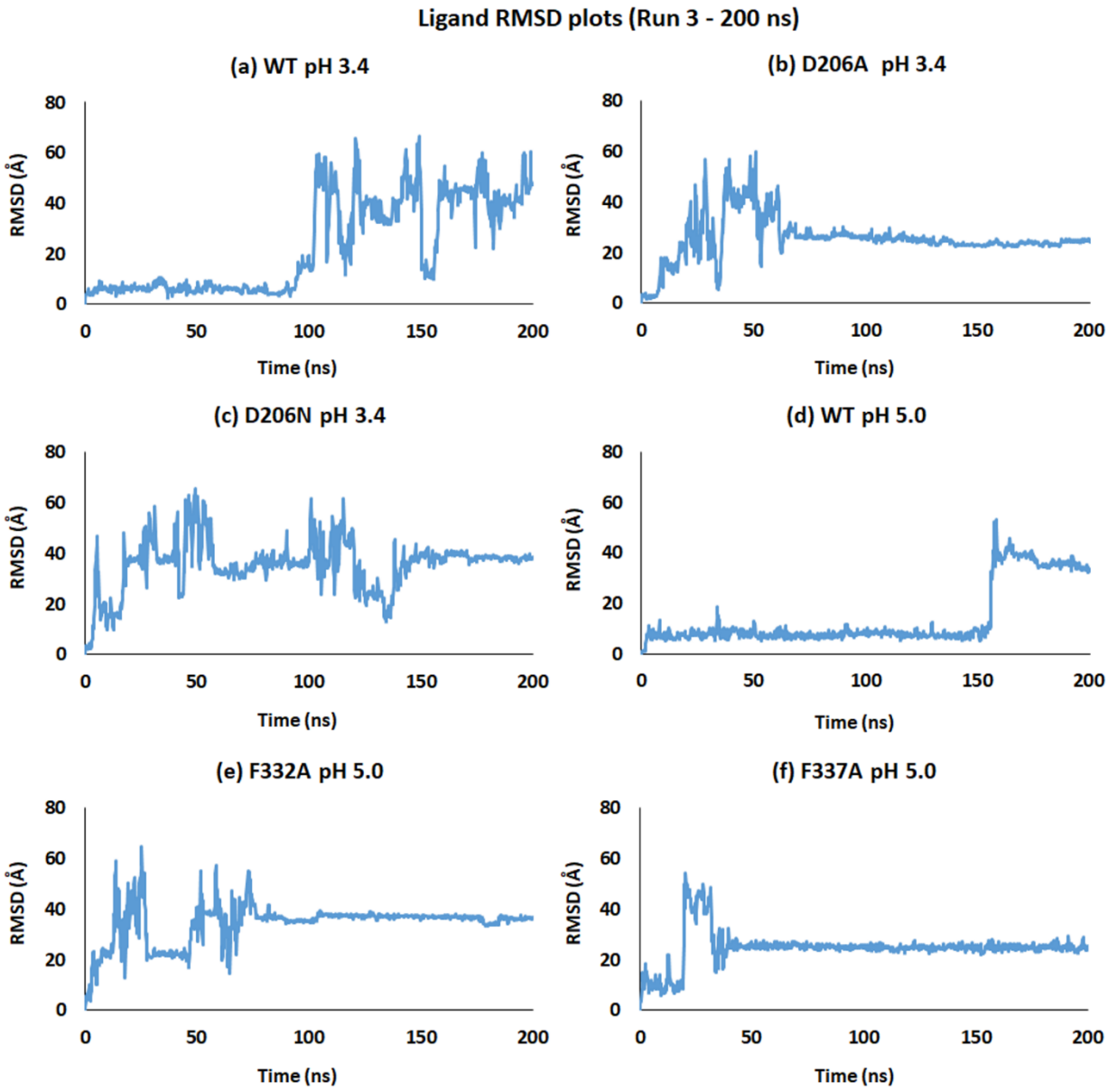


Figure S11. RMSD plots of 2,6-DMP during run 3 (200 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f)** F337A (pH 5.0).

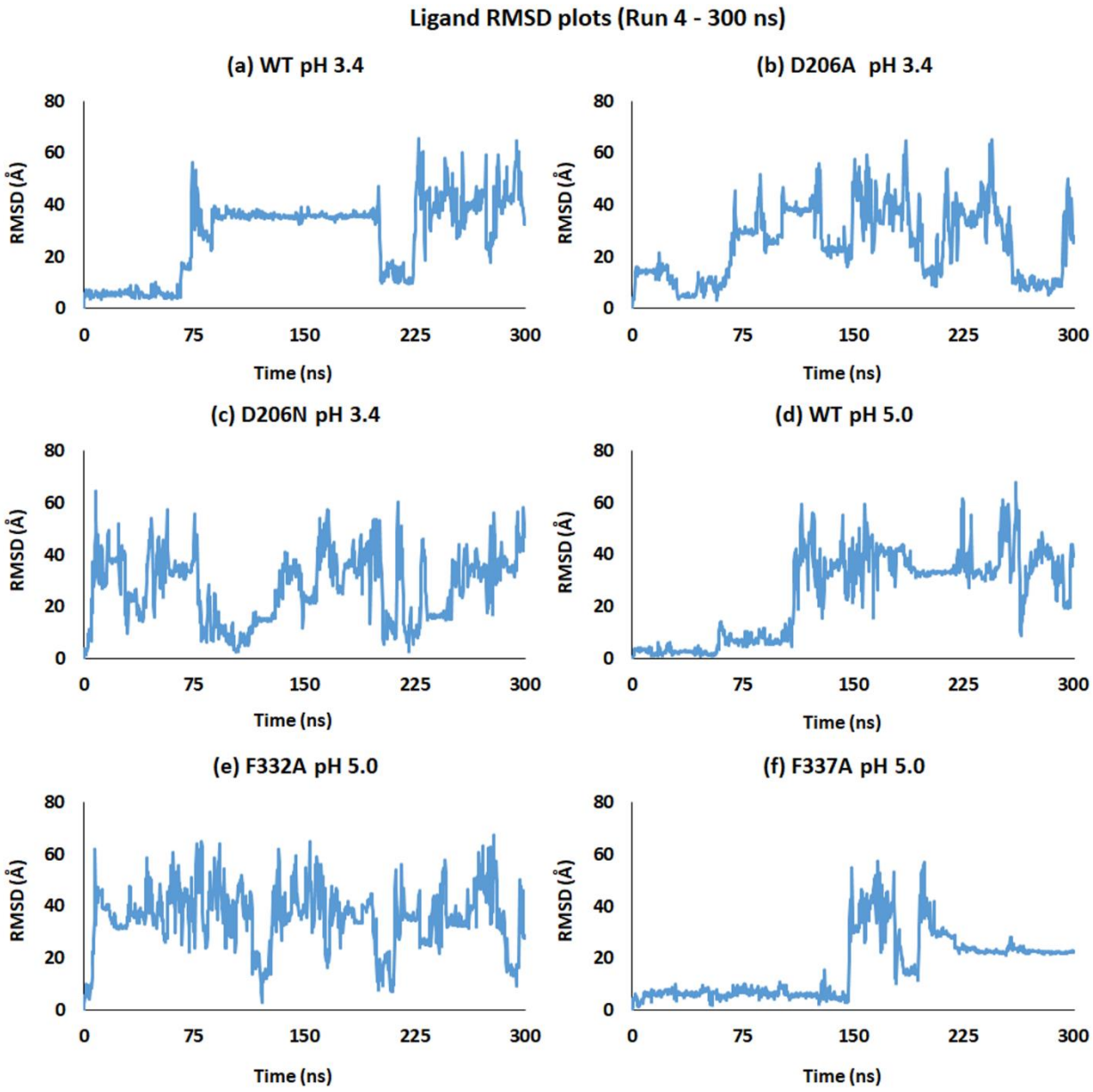


Figure S12. RMSD plots of 2,6-DMP during run 4 (300 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).

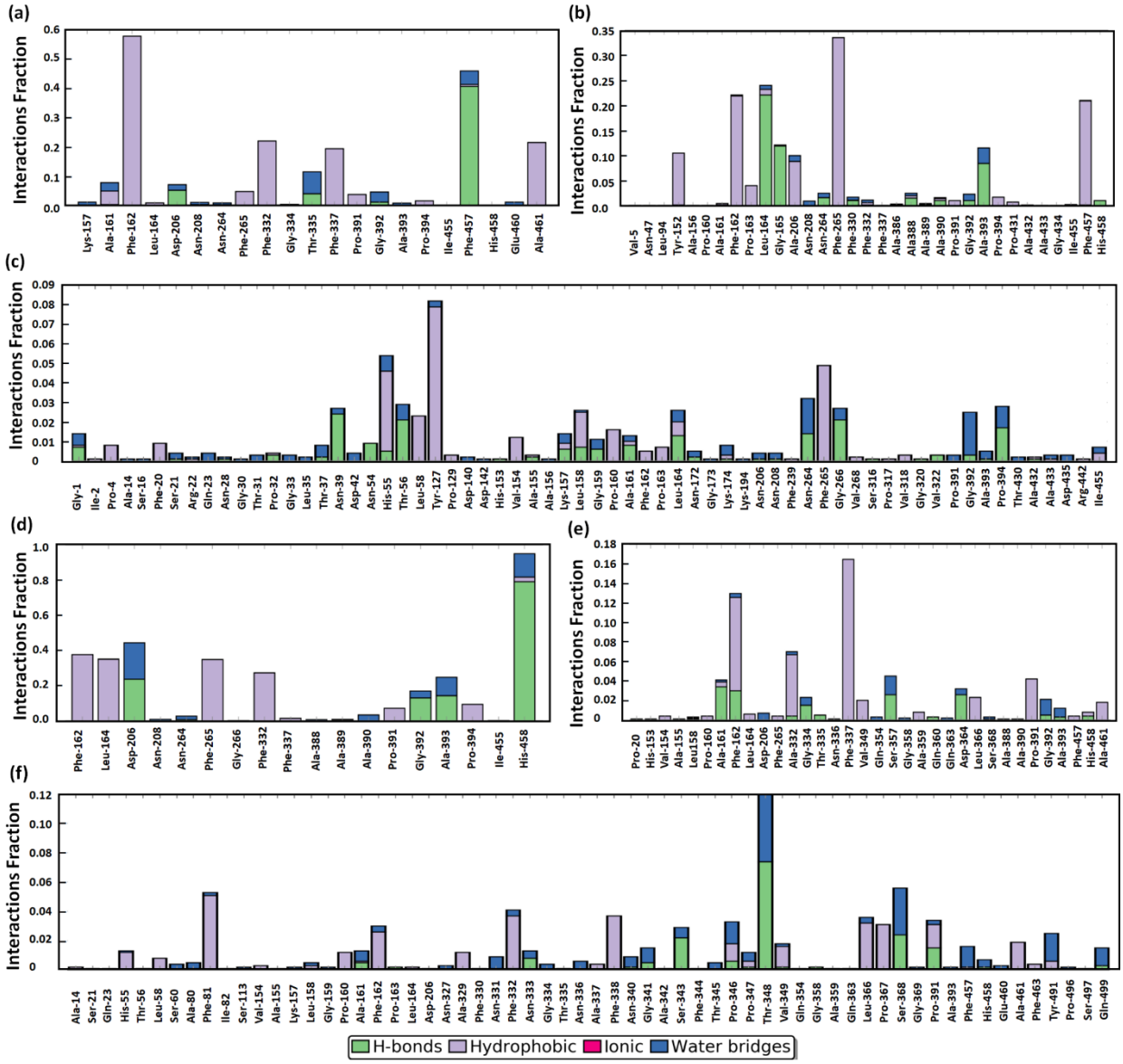


Figure S13. Interactions fraction of all the six laccase variants with 2,6-DMP during 50 ns MD simulations. **(a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f)** F337A (pH 5.0).

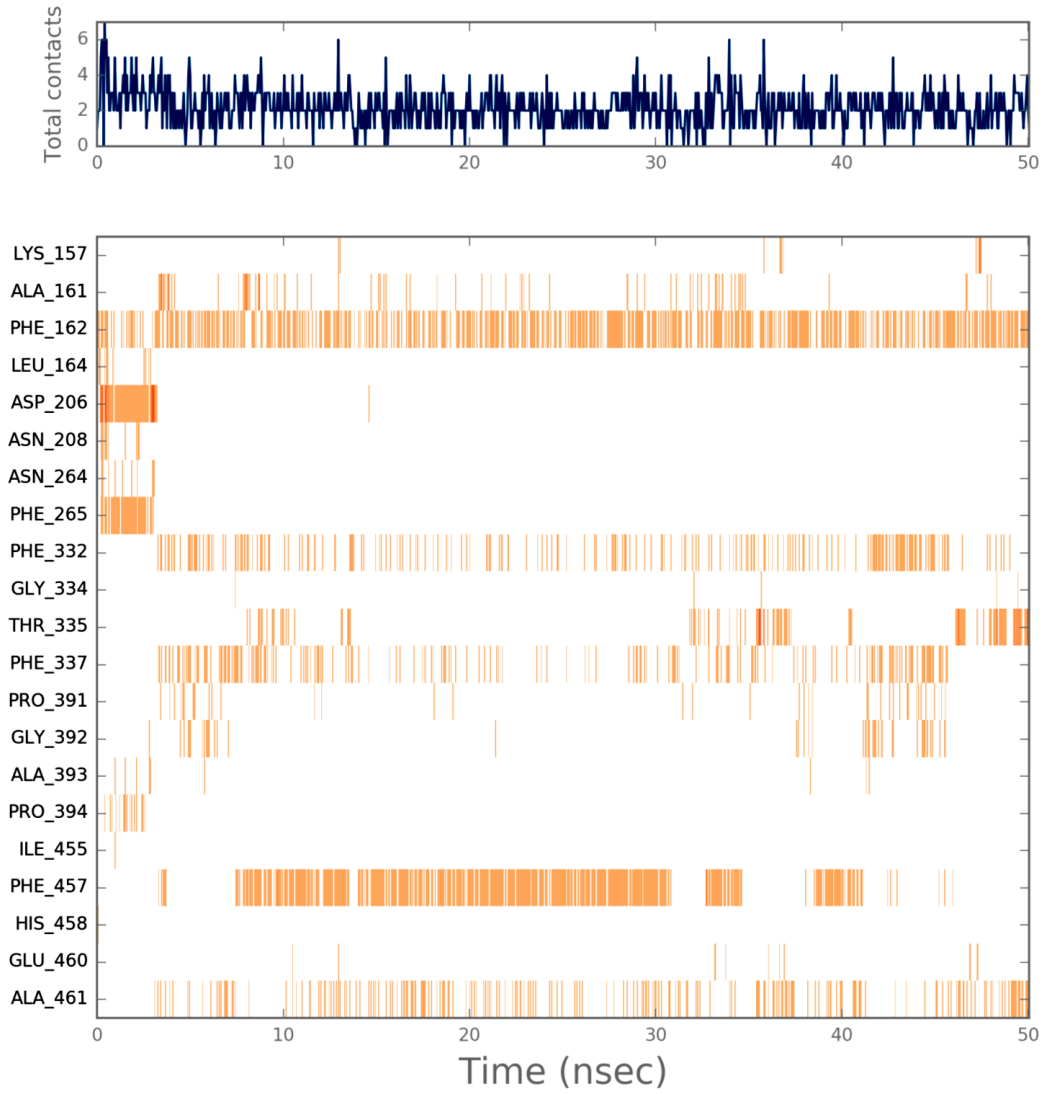


Figure S14. Timeline representation of the laccase WT (pH 3.4) and 2,6-DMP contacts and interactions during 50 ns MD run.

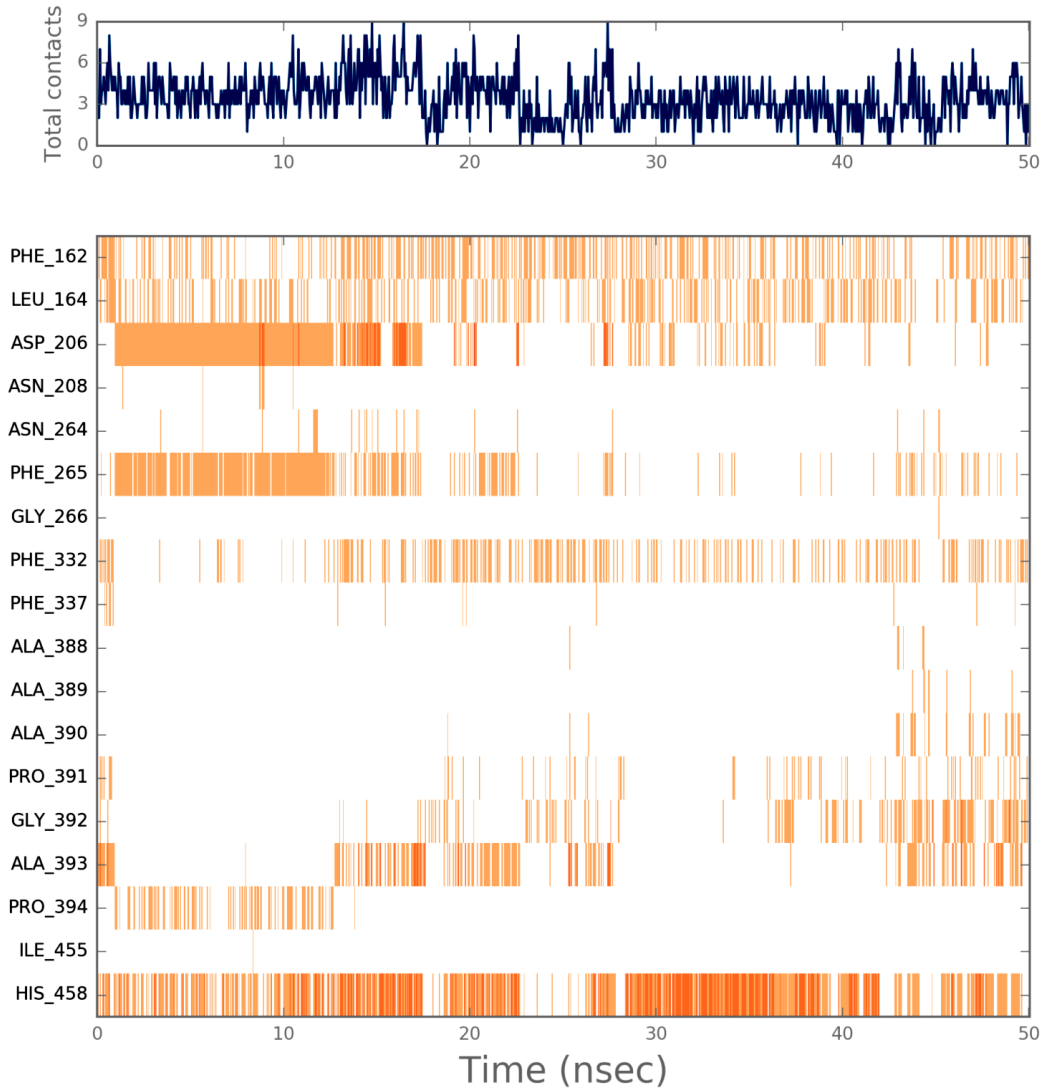


Figure S15. Timeline representation of the laccase WT (pH 5.0) and 2,6-DMP contacts and interactions during 50 ns MD run.

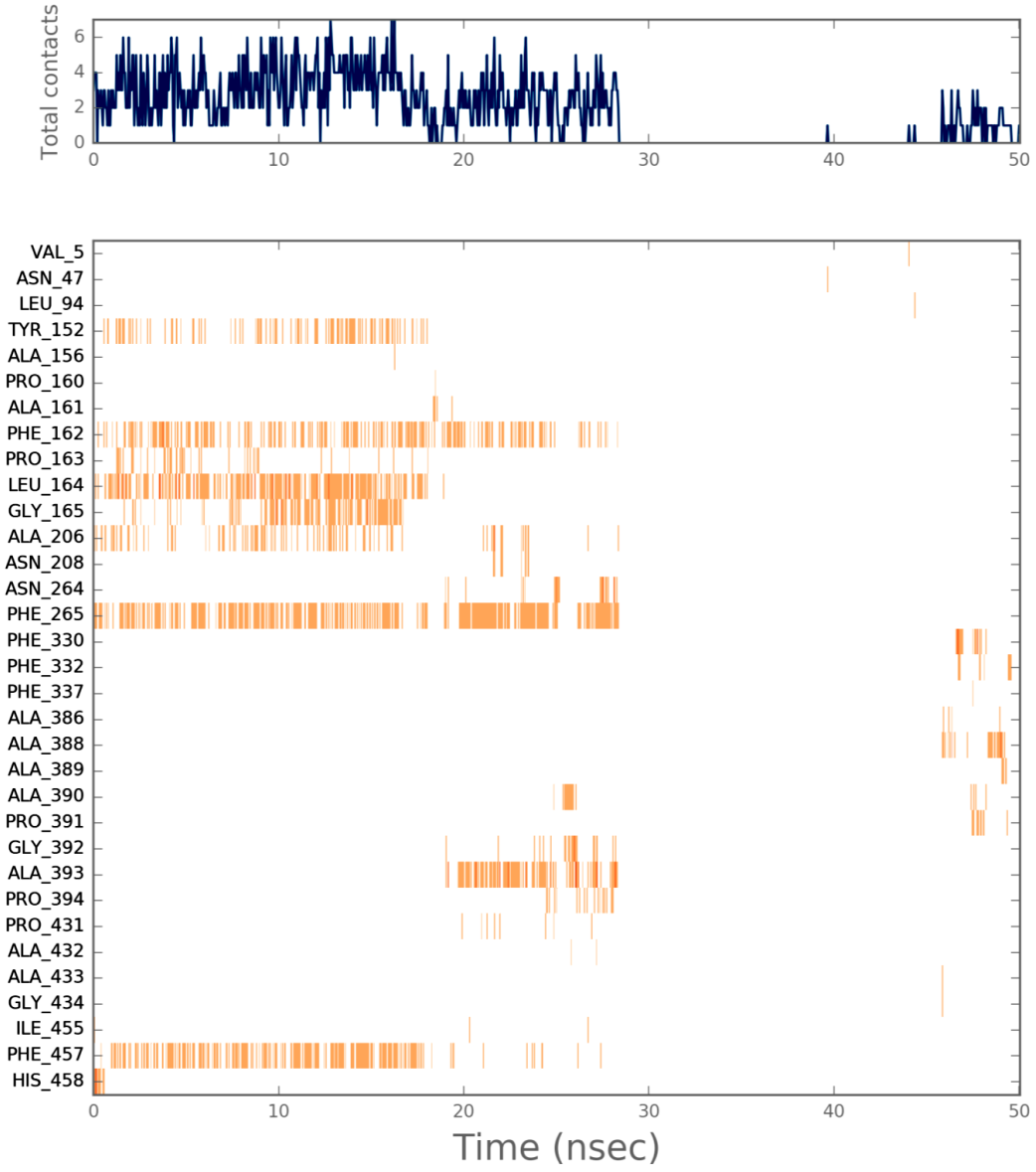


Figure S16. Timeline representation of the laccase D206A (pH 3.4) and 2,6-DMP contacts and interactions during 50 ns MD run.

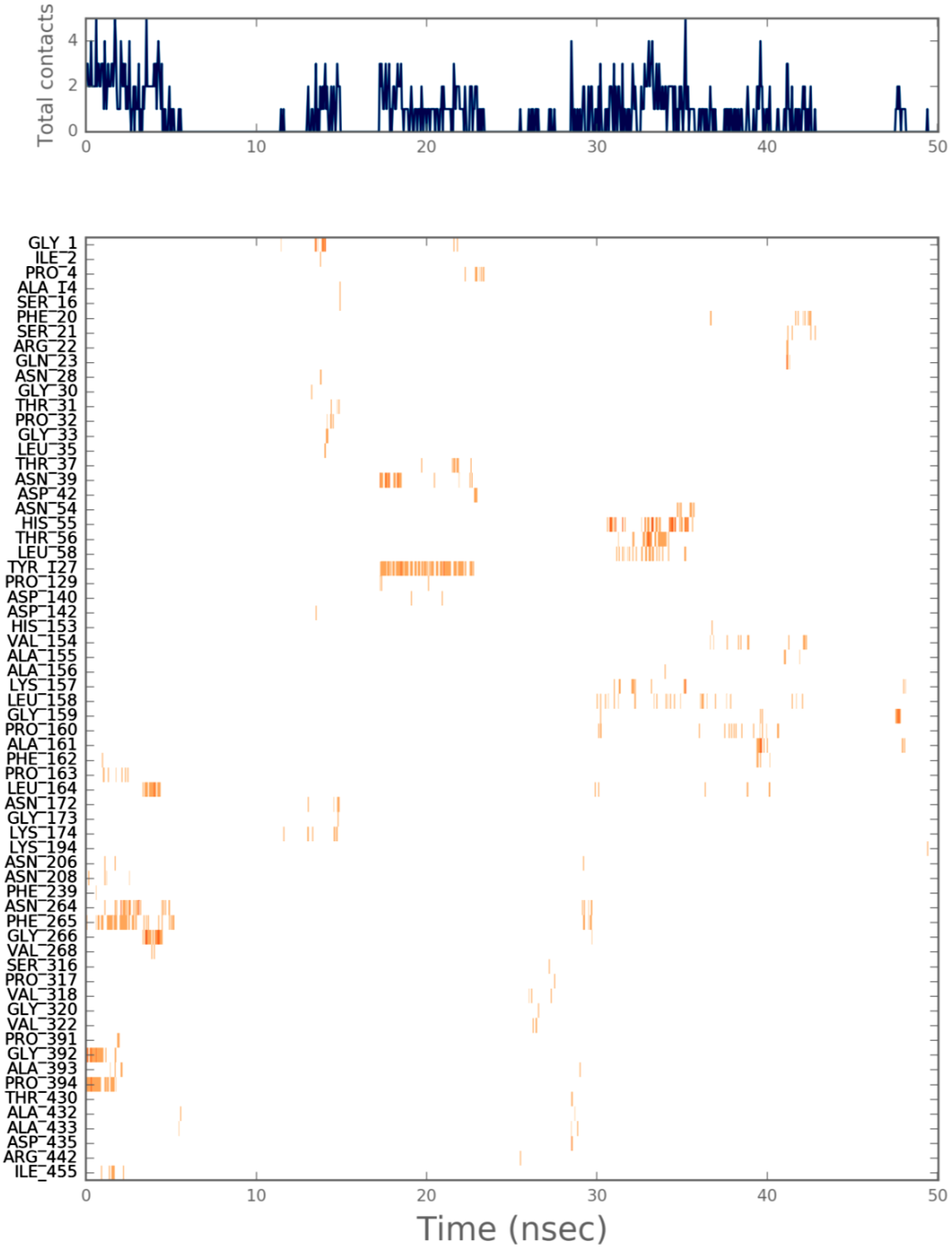


Figure S17. Timeline representation of the laccase D206N (pH 3.4) and 2,6-DMP contacts and interactions during 50 ns MD run.

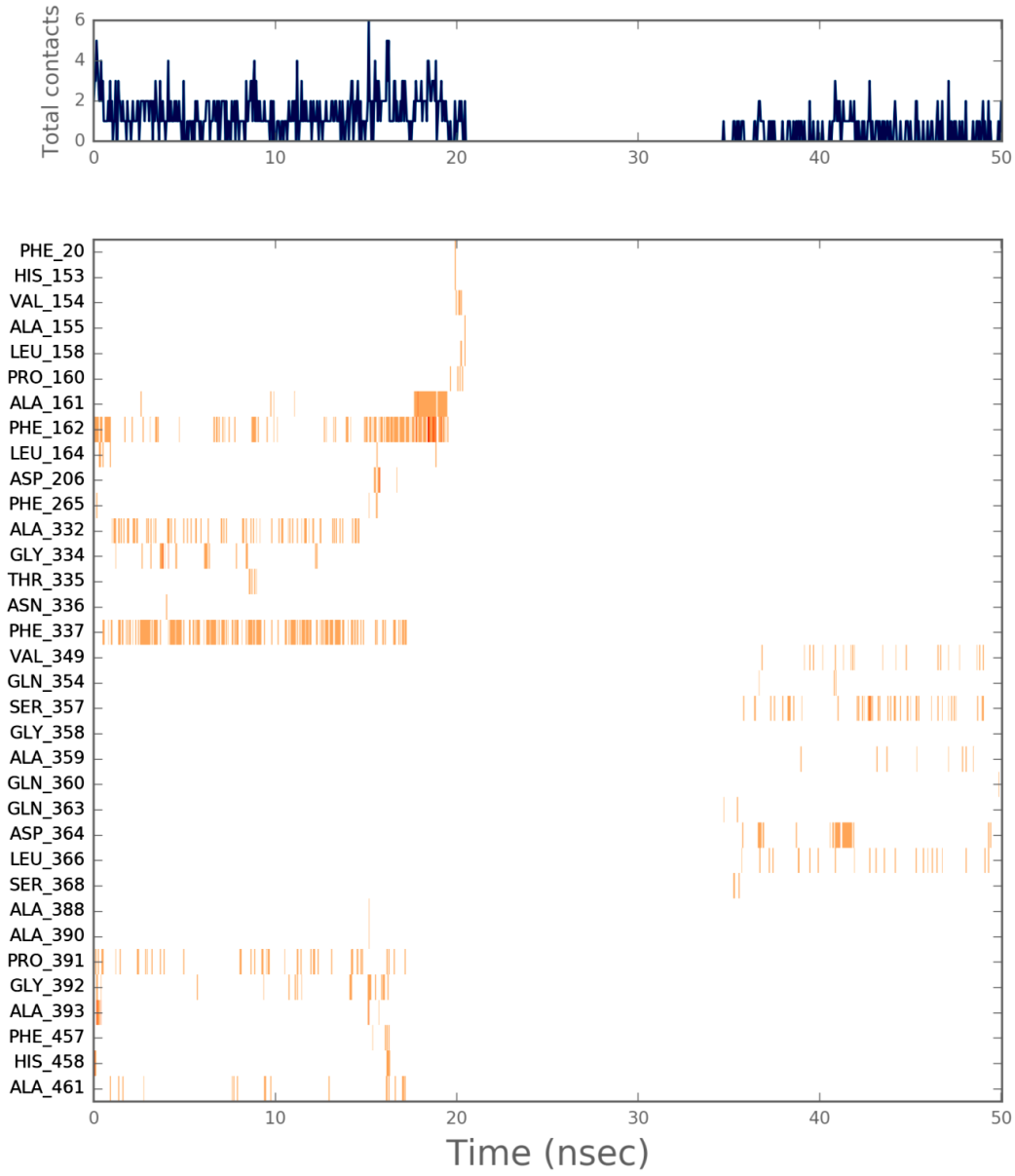


Figure S18. Timeline representation of the laccase F332A (pH 5.0) and 2,6-DMP contacts and interactions during 50 ns MD run.

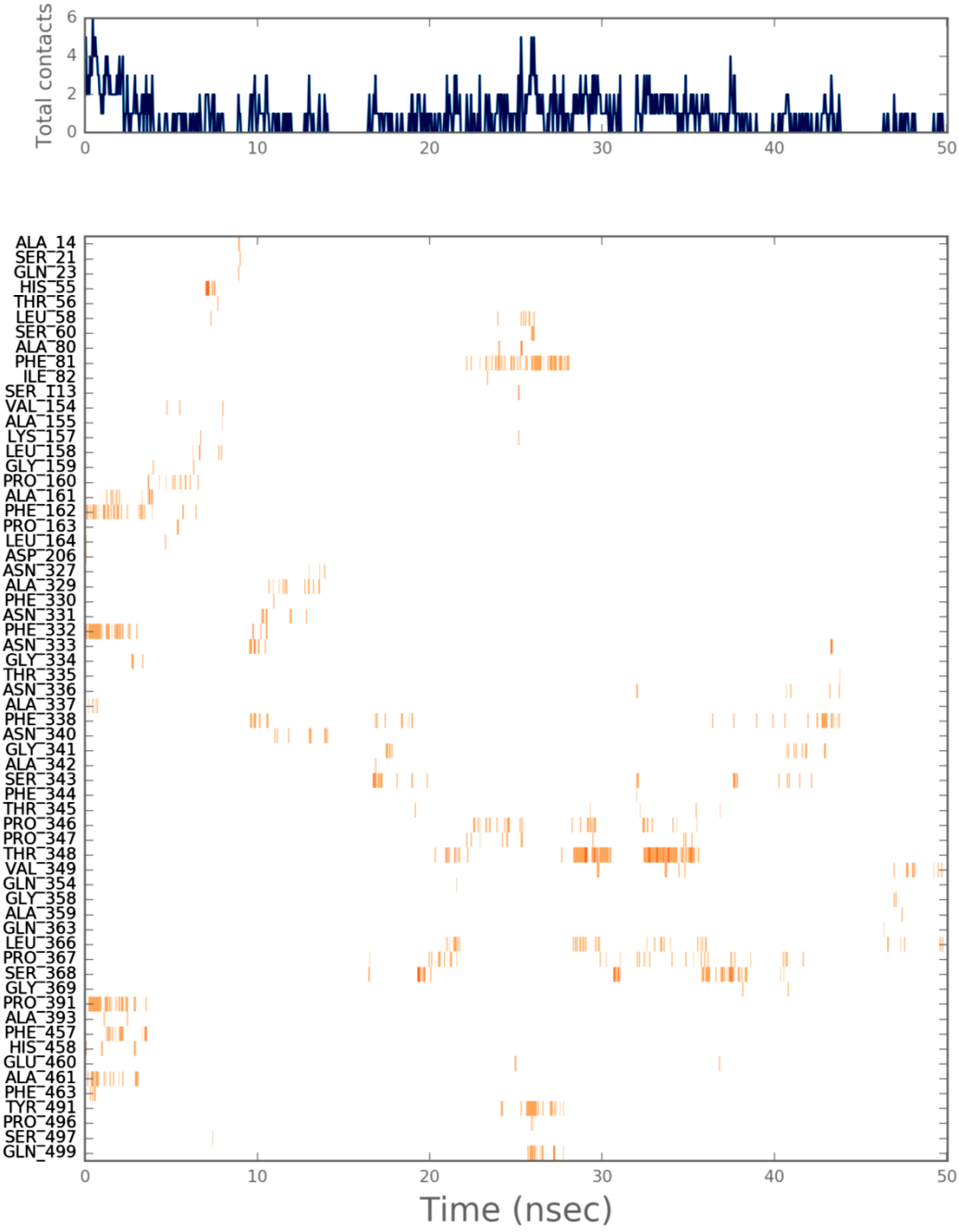


Figure S19. Timeline representation of the laccase F337A (pH 5.0) and 2,6-DMP contacts and interactions during 50 ns MD run.

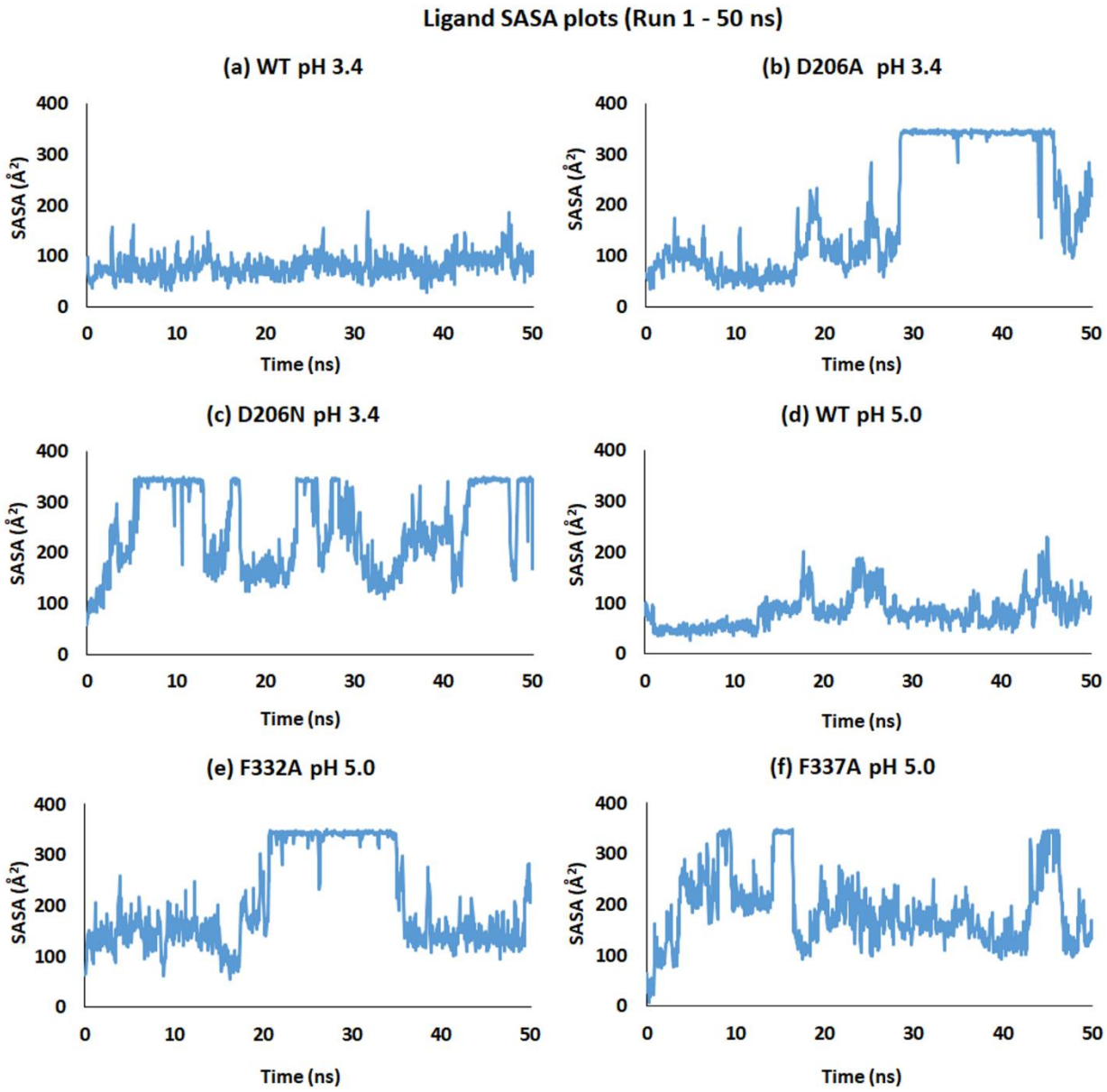
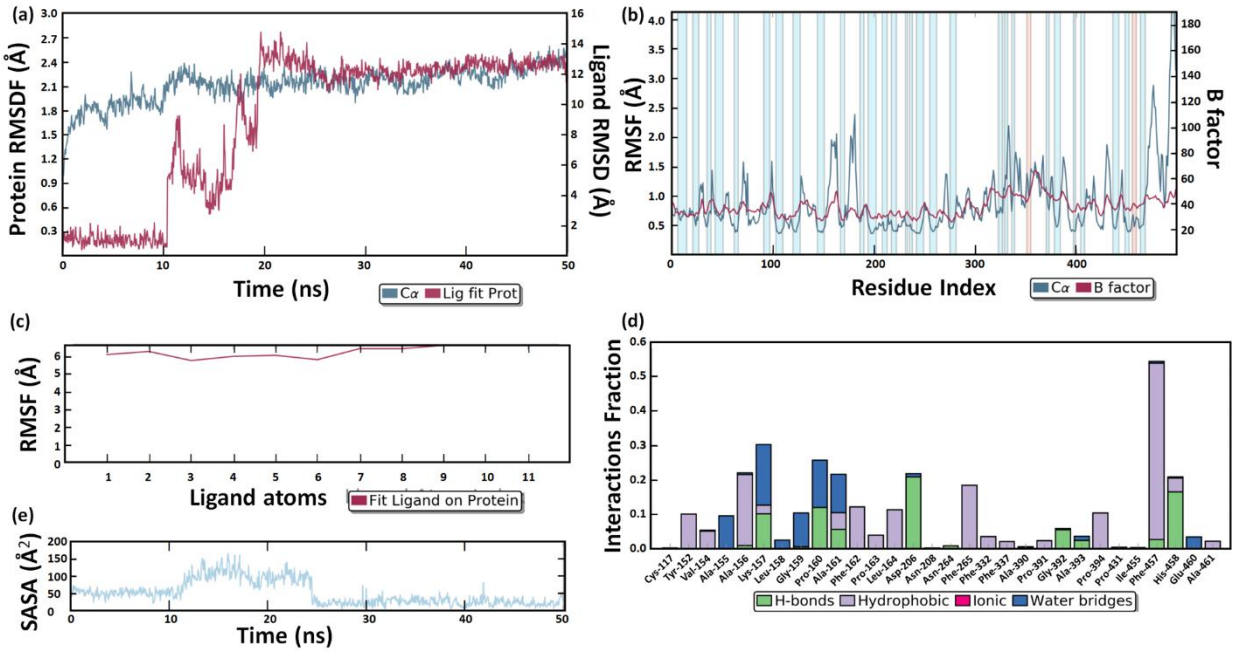


Figure S20. SASA plots of 2,6-DMP during run 1 (50 ns) MD simulations. (**a)** WT (pH 3.4). **(b)** D206A (pH 3.4). **(c)** D206N (pH 3.4). **(d)** WT (pH 5.0). **(e)** F332A (pH 5.0). **(f**) F337A (pH 5.0).



Figures S21. MD simulation (for 50 ns) of another pose of 2,6-DMP in the TvL WT structure (pH 3.4). **(a)** Protein and ligand RMSD plot. **(b)** Protein RMSF and B factor. Red regions represent α helix, blue color shows β strand, and white color indicate the loop regions that occurred >70% simulation time. **(c)** Ligand RMSF. **(d)** Protein-ligand interactions. **(e)** Ligand SASA.

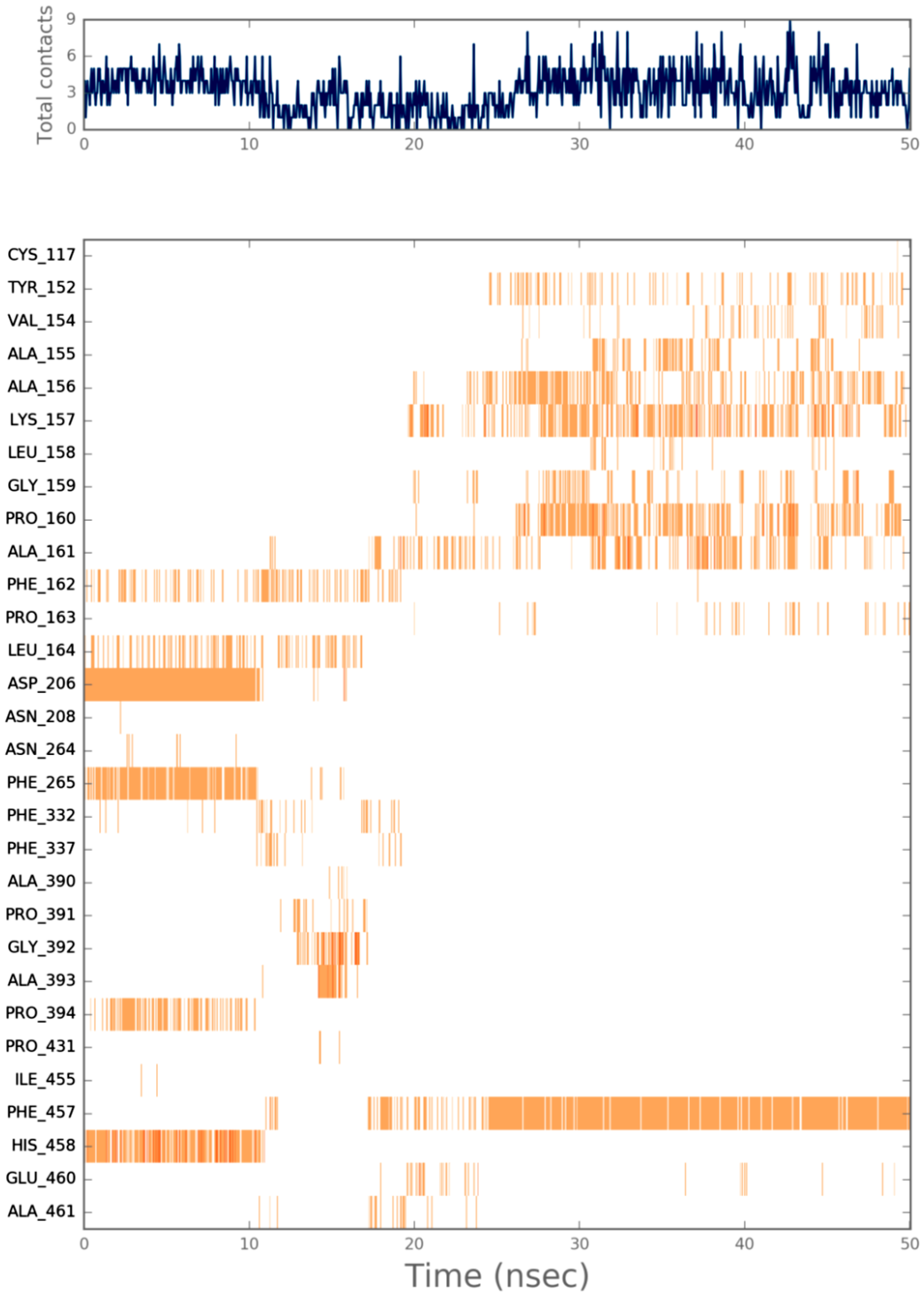
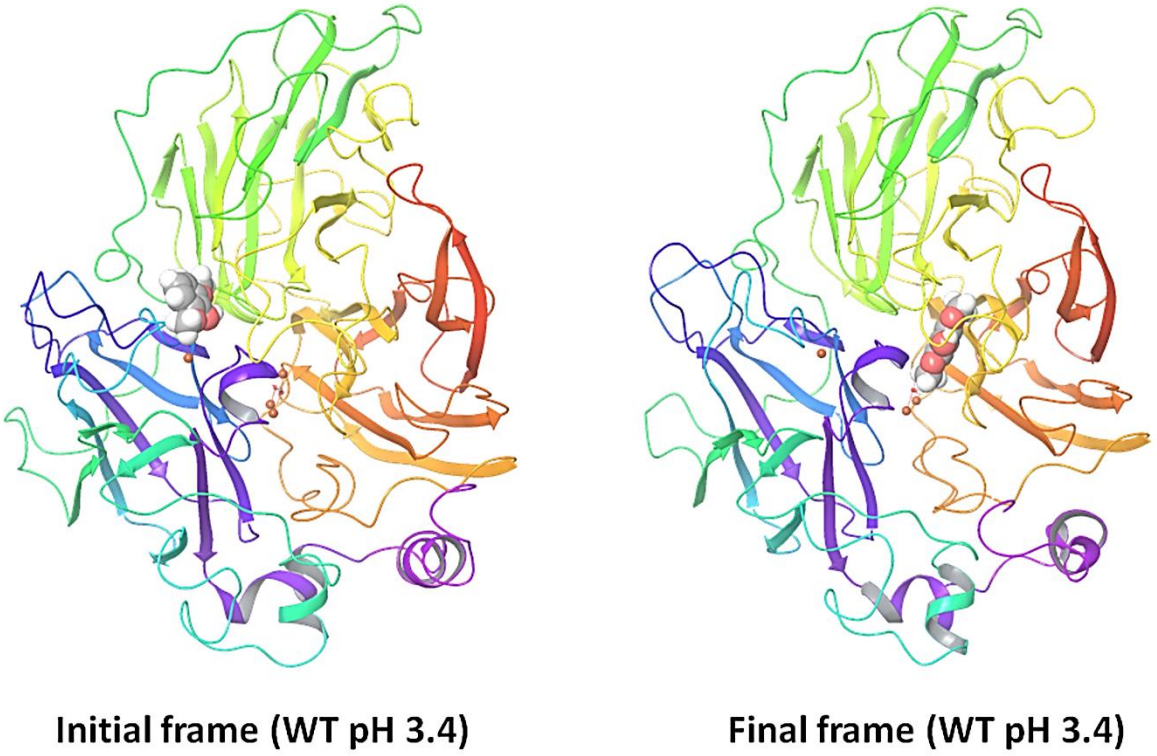


Figure S22. Timeline representation of the laccase WT (pH 3.4) and 2,6-DMP pose contacts and interactions during 50 ns MD run.



Figures S23. Initial and final frame structures of a 50-ns MD simulation of another pose of 2,6-DMP in the TvL WT structure (pH 3.4).

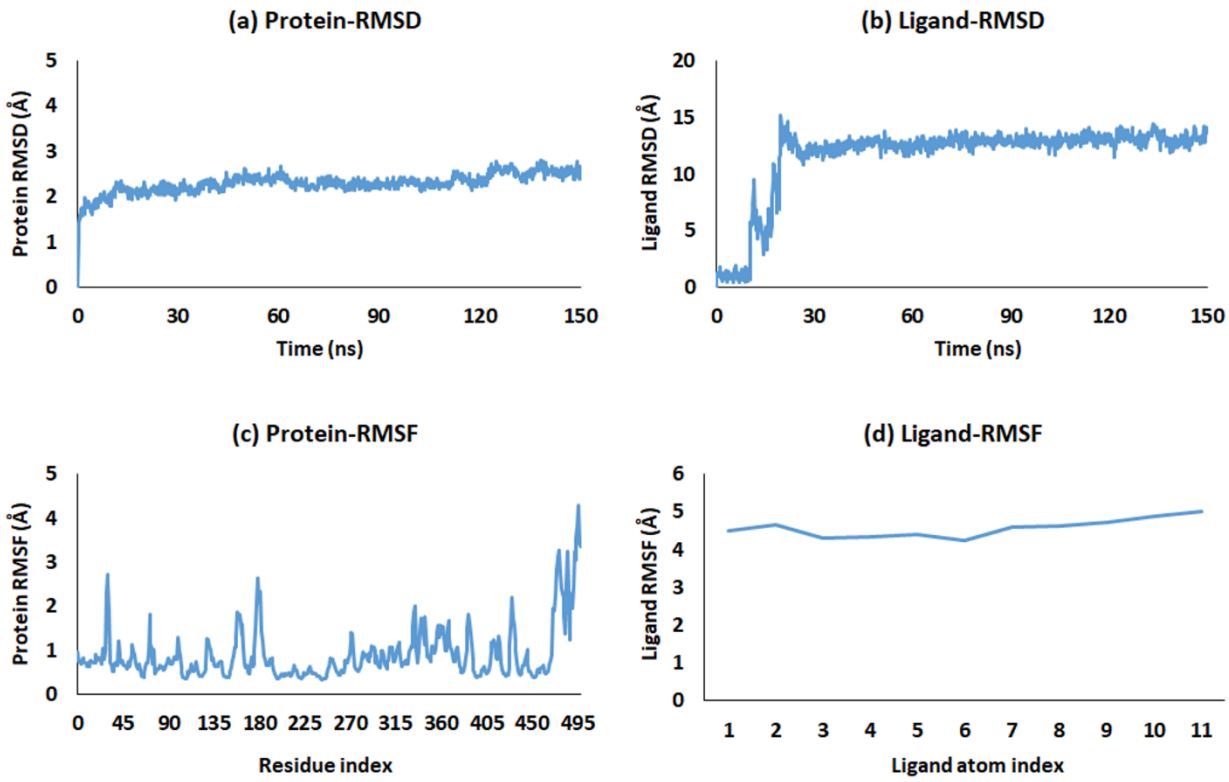


Figure S24. MD simulation (for 150 ns) of another pose of 2,6-DMP in the TvL WT structure (pH 3.4). **(a)** Protein RMSD plot. **(b)** Ligand RMSD plot. **(c)** Protein RMSF plot. **(d)** Ligand RMSF plot.

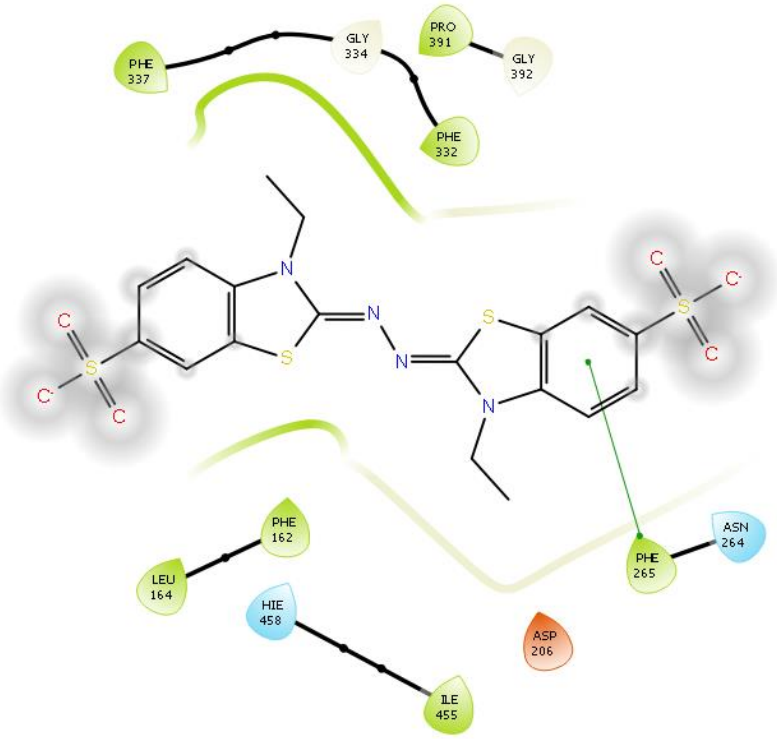


Figure S25. ABTS interaction with WT (pH 3.4) TvL.