

# Supplemental Information: Effects of Ionic Liquids on Laccase from *Trametes Versicolor*

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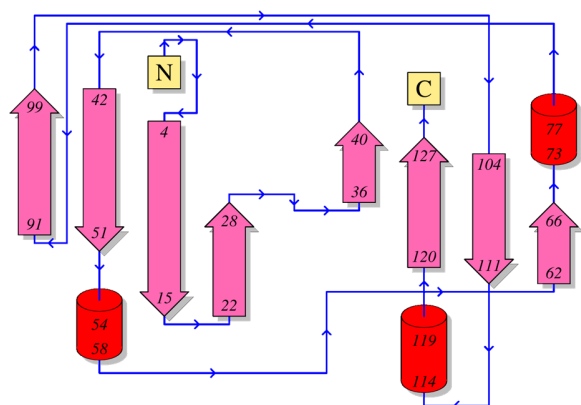
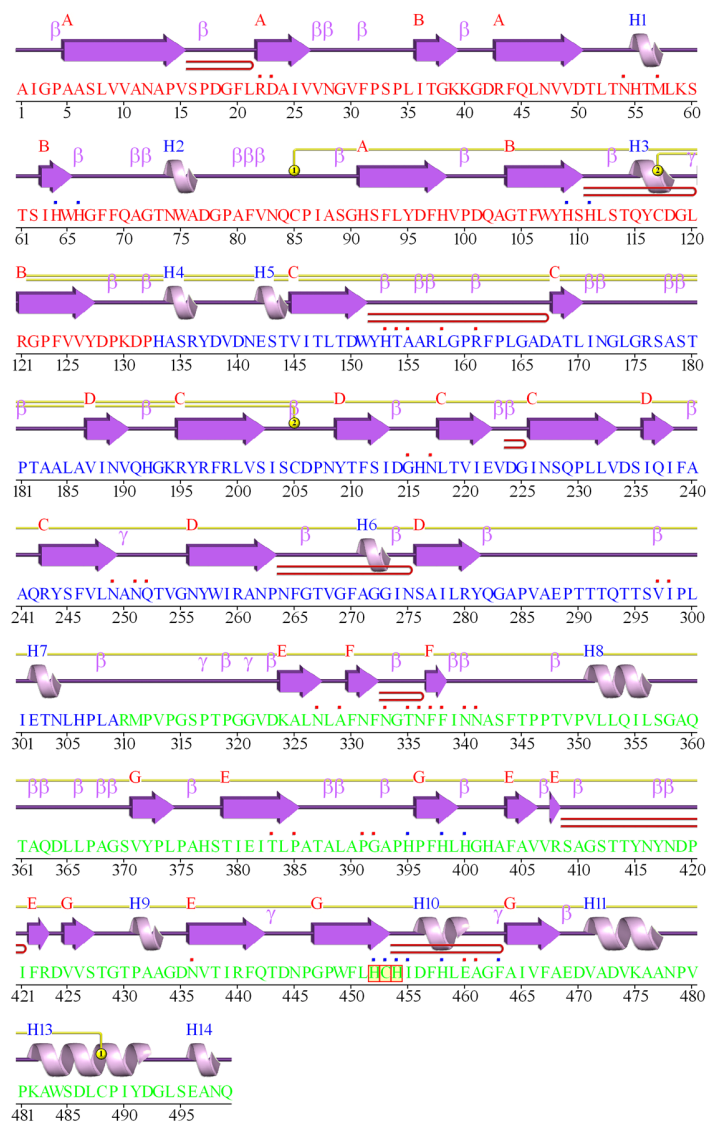
*Gregory A Caputo*

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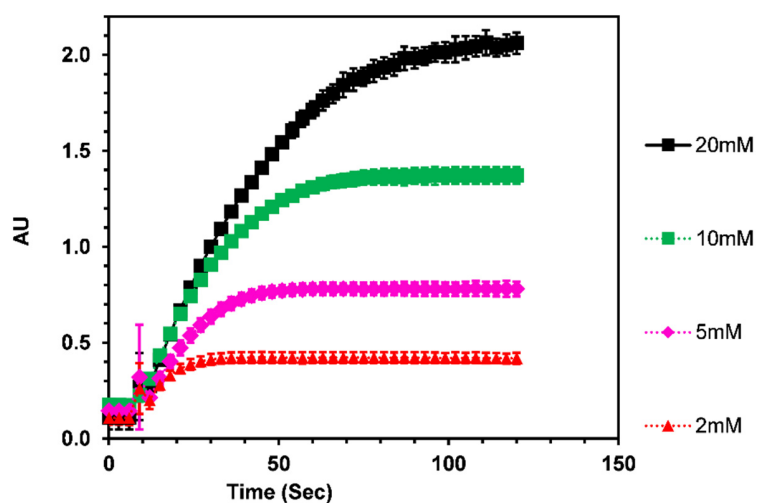
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*Rowan University*

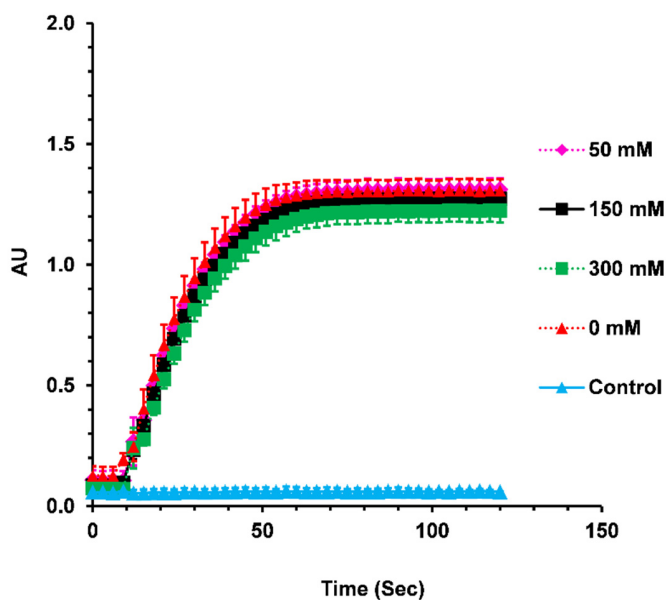
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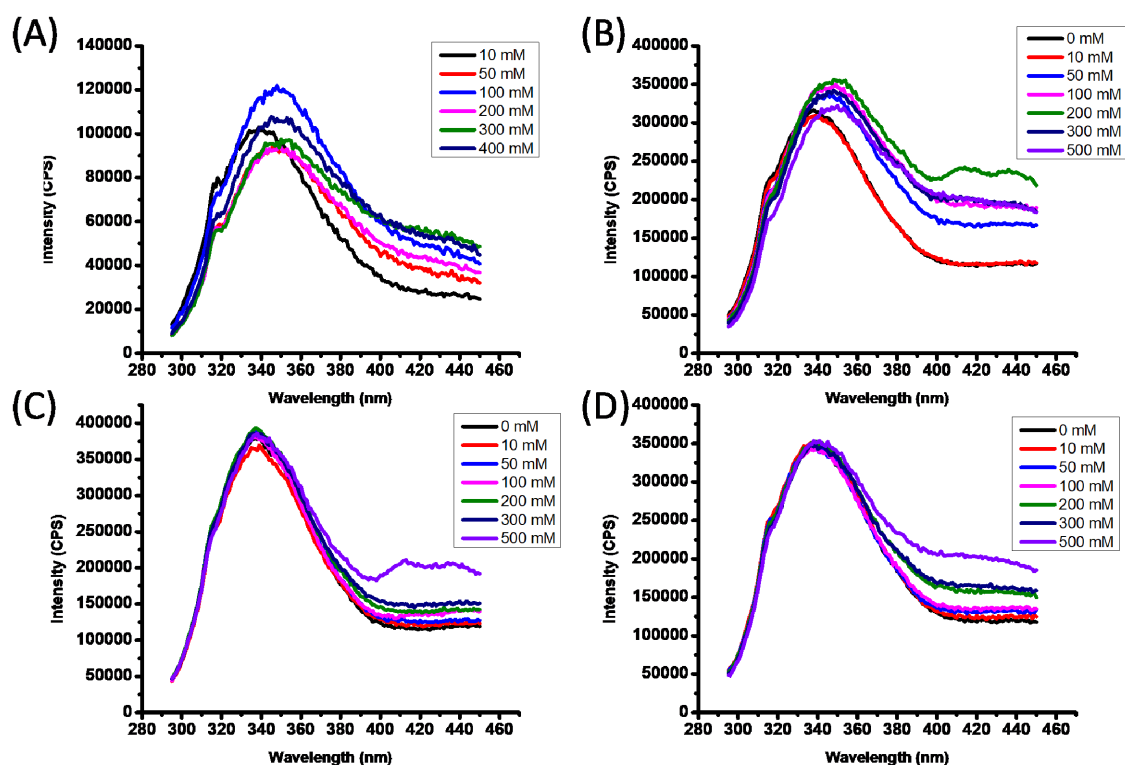
**Supplemental Figure S1.** Primary structure, secondary structure and topology of *T. versicolor* laccase protein (PDB ID: 1GYC) from the PDBsum server.



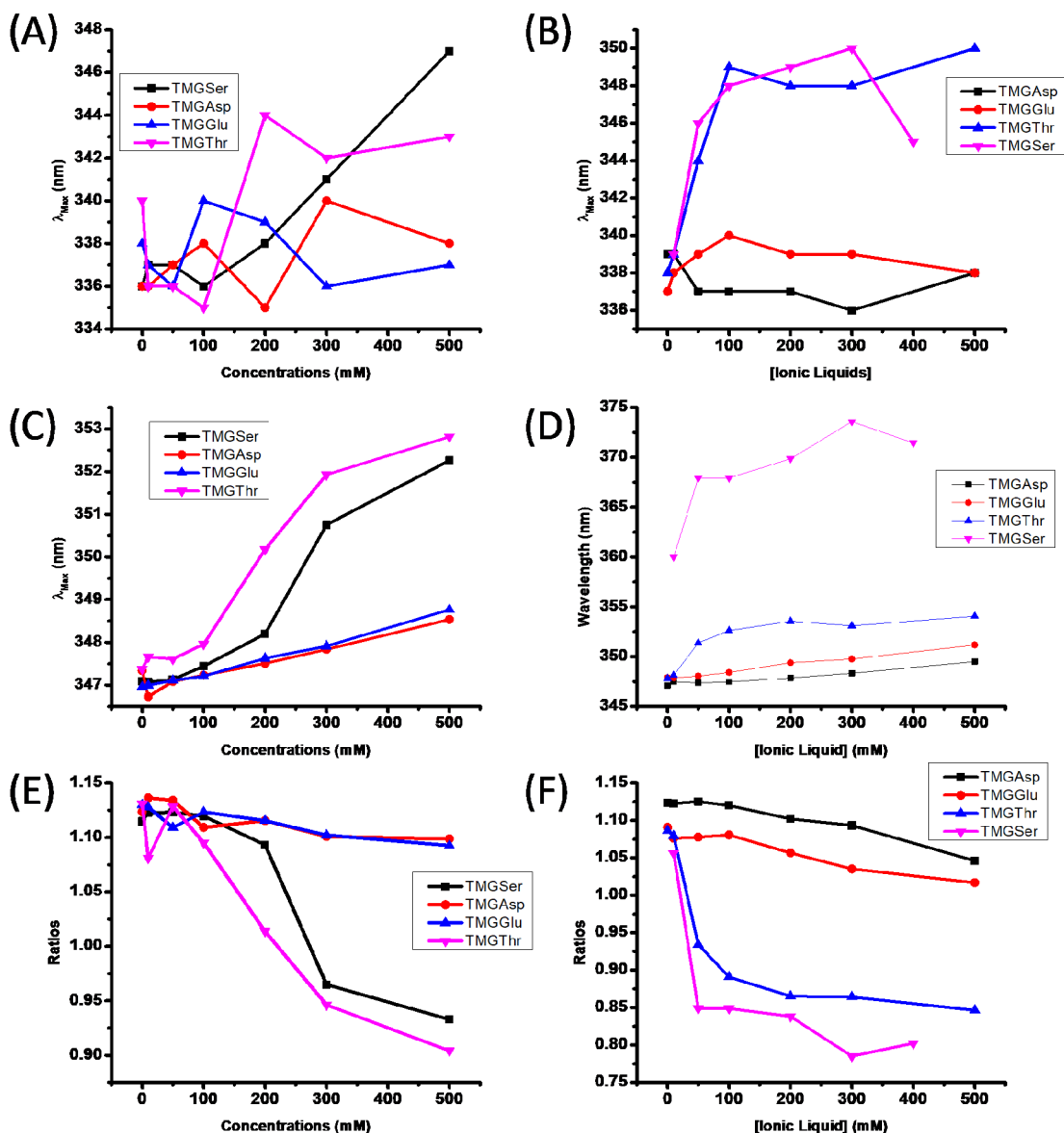
**Supplemental Figure S2** – Laccase enzymatic activity with variable substrate concentration. Conversion of ABTS by laccase at pH 5.1 was monitored over time. ABTS concentrations are shown in the legend. Data points are the average of 3-7 replicates and error bars represent standard deviations.



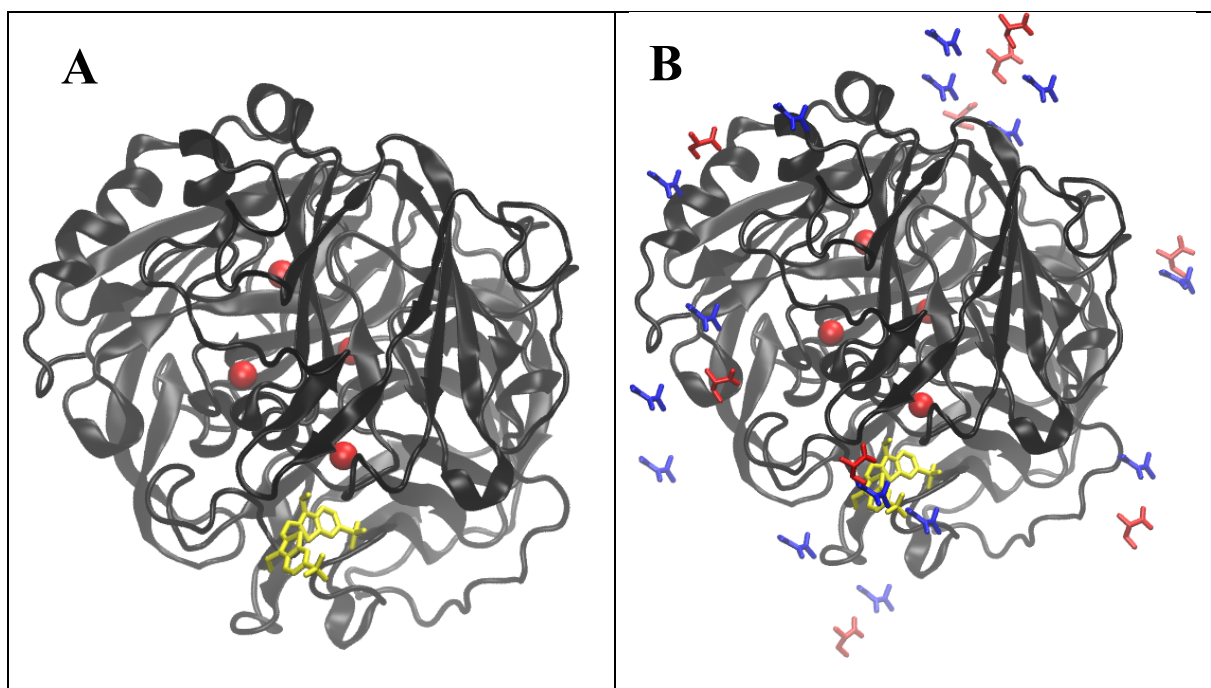
**Supplemental Figure S3** - Laccase enzymatic activity in the presence of free serine. Conversion of ABTS by laccase at pH 5.1 was monitored over time. ABTS concentrations were constant in all samples. Serine concentrations for each sample are shown in the legend. The control data refers to samples that contained laccase and 300 mM serine but no ABTS. Data points are the average of 3-7 replicates and error bars represent standard deviations.



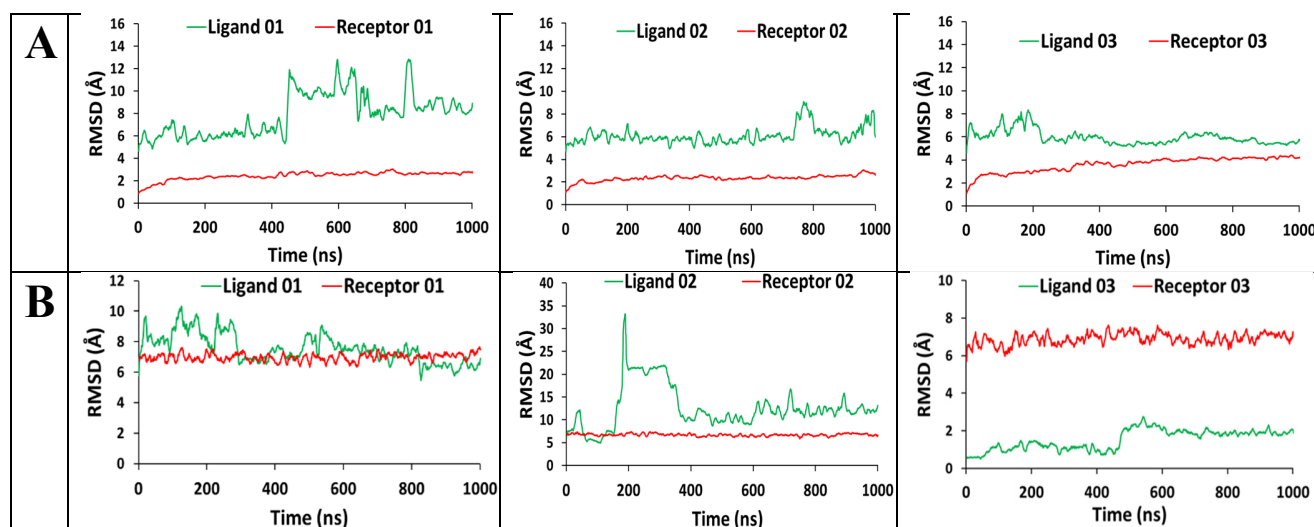
**Supplemental Figure S4** Laccase fluorescence emission spectra at pH 7.1 in the presence and absence of amino acid ionic liquids. In all panels, laccase concentration was 6.25  $\mu$ M and amino acid IL concentrations are denoted in the legend. Fluorescence was measured in the presence of (A) TMG-Ser, (B) TMG-Thr, (C) TMG-Asp, and (D) TMG-Glu. Representative spectra are shown in each panel. Ionic liquid concentrations are shown in the legends



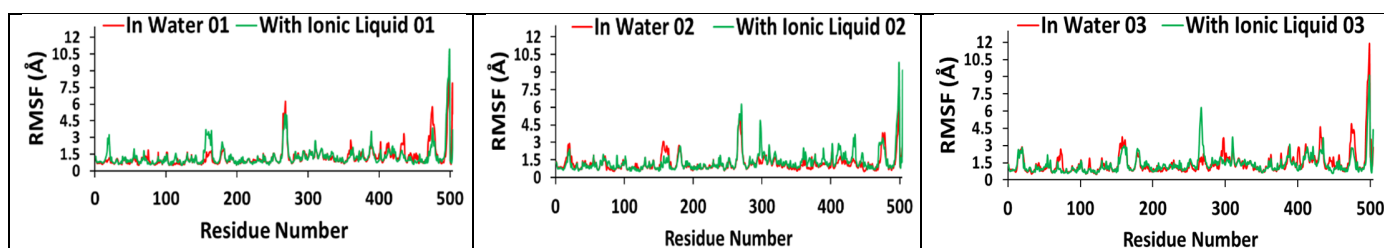
**Supplemental Figure S5** Laccase fluorescence emission spectral properties at pH 5.1 or 7.1 in the presence and absence of amino acid ionic liquids. In all panels, laccase concentration was 6.25 mM and amino acid IL identities are denoted in the legends. Fluorescence properties measured were (A)  $\lambda_{\text{max}}$  at pH 5.1, (B)  $\lambda_{\text{max}}$  at pH 7.1, (C) spectral barycenter at pH 5.1, (D) spectral barycenter at pH 7.1, (E)  $I_{350}/I_{330}$  at pH 5.1, and (F)  $I_{350}/I_{330}$  at pH 7.1.



**Supplemental Figure S6:** Laccase and ABTS in water (**A**) and in water with TMG-Ser (**B**). Protein backbone: Grey ribbons. ABTS ligand: Licorice tubes in yellow.  $\text{Cu}^{2+}$  ions: red VDW balls. T1  $\text{Cu}^{2+}$  ion (left) is adjacent to ABTS; T2  $\text{Cu}^{2+}$  ion (right) is parallel across from T1  $\text{Cu}^{2+}$  ion. T3  $\text{Cu}^{2+}$  ions (top and bottom) are perpendicular to the T1 and T2  $\text{Cu}^{2+}$  ions. TMG: licorice in blue; Ser: licorice in red.

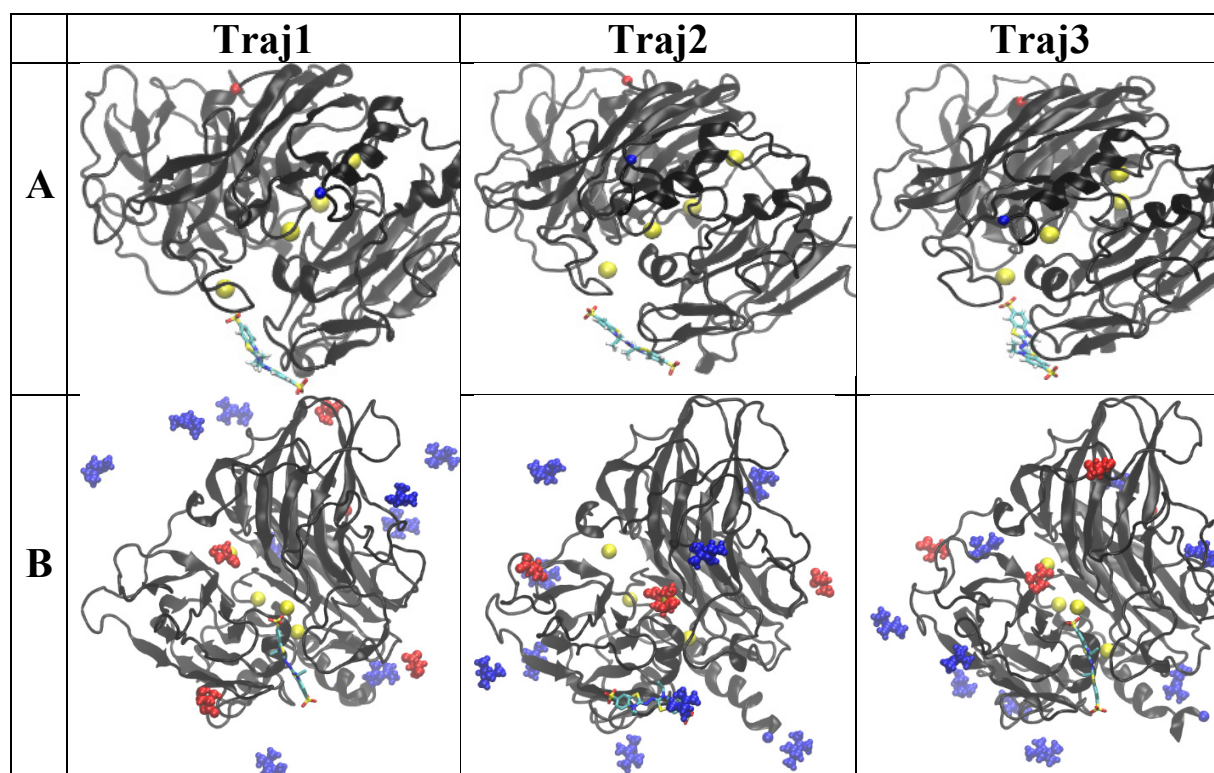


**Supplemental Figure S7.** RMSD of heavy ligand atoms (green) and laccase protein  $\alpha$  atoms (red) over three 1000 ns trajectories. (A) In water. (B) In water with TMG-Ser.

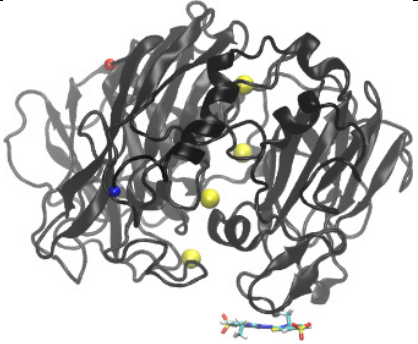
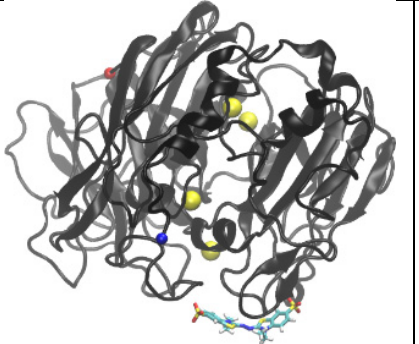
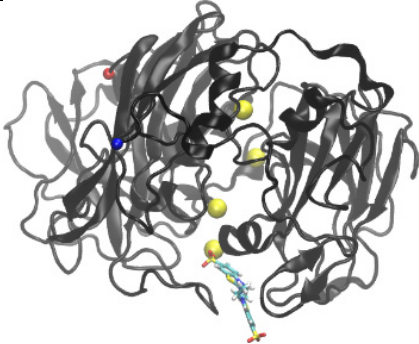
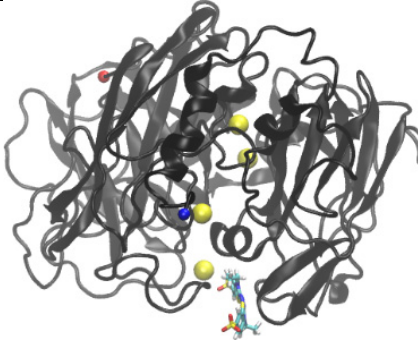
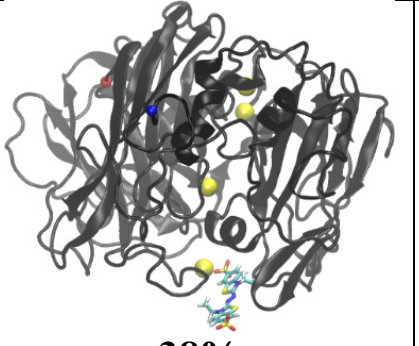
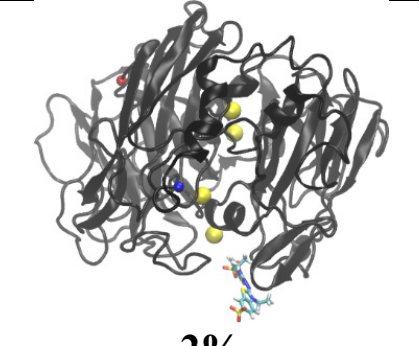


**Supplemental Figure S8:** RMSF of laccase protein residues (1 to 504) from three 1000 ns trajectories in water (red) and in water with TMG-Ser (green).

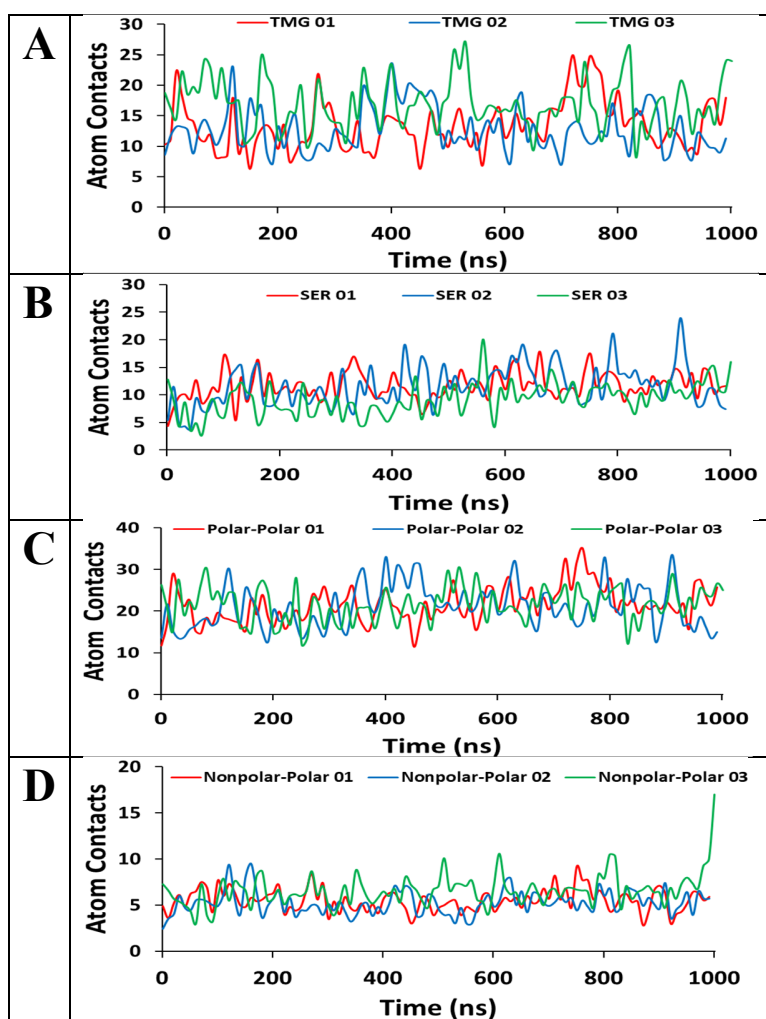




**Supplemental Figure S9:** Last snapshot of three 1000 ns trajectories of laccase protein with ligand ABTS. (A) In water. (B) In water with TMG-Ser.  $\text{Cu}^{2+}$  ion: yellow VDW ball; Ser anion: red VDW ball; TMG cation: blue VDW ball; N-terminus: red VDW ball; C-terminus: blue VDW ball.

	Cluster 1	Cluster 2	Cluster 3
A	 <p>46%</p>	 <p>23%</p>	 <p>23%</p>
B	 <p>59%</p>	 <p>38%</p>	 <p>2%</p>

**Supplemental Figure S10:** Most abundant clusters with percentage abundance of three 1000 ns trajectories of laccase protein with ligand. (A) In water. (B) In water with TMG-Ser.



**Supplemental Figure S11:** Atom contact plots for laccase protein in TMG-Ser over three 1000 ns trajectories. (A) Protein in TMG. (B) Protein in Ser. (C) Polar Protein in TMG and Ser. (D) Hydrophobic Protein in TMG and Ser. Red, blue and green lines represent trajectories 1, 2 and 3, respectively.

**Table S1:** Molecular dynamics simulations of Laccase and ABTS with/without IL.

ID	Solvent/IL	#of runs	# of Water molecules	# of TMG ions	# of Ser Ions	Box Size (Å)*	NPT eq. (ns)	NVT (ns)	Total time (ns)
1	Water	3	7778	0	0	77.7	1	999	1000
2	Water/TMG-Ser	3	7788	15	9	78.6	1	999	1000

\*Triclinic box equivalent to the true truncated octahedron box shape.

**Table S2:** Average IL-protein contacts for the laccase system containing IL (TMG-Ser).

Average Contacts (last 200 ns)			
Protein-TMG	Protein-Ser	Polar-IL	Hydrophobic-IL
14±4	12±3	22±2	6±1

**Table S3.** Average RMSF values of laccase protein in water and with/without TMG-Ser.

System	RMSF (Å)
Water	1.3 ± 0.8
Water/TMG-Ser	1.3 ± 0.9