# Supporting information

## Supplementary material and methods

1. **Molecular docking followed by molecular dynamics simulations of polyphenols with different SOD1 structures**

   The structures of polyphenols, WT SOD1, apo SOD1, $SOD1^{SH}$, and Zn-$SOD1^{SH}$ were obtained as described in the main methodology section. The docking of polyphenols with protein was carried out using AUTODOCK followed by 500 ns MD simulations production run. The simulations were performed as described earlier.

2. **Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) calculation for calculating binding energies**

The MM/PBSA method was used to calculate and compare the binding energies of protein-ligand complexes based on MD simulation trajectory data. For carrying out MM/PBSA calculation a method developed by Kumari et al. was used which require a single trajectory of protein-ligand complex and can be easily plugged into GROMACS. Here, total binding energy equation can be written as:

$$\Delta G_{BE} = \Delta E_{MM} - TS_{MM} + \Delta G_{solv} \quad (1)$$

Where $\Delta G_{BE}$ is the binding free energy change, $\Delta E_{MM}$ is molecular mechanics energy change, $\Delta G_{solv}$ is solvation free energy change, and $TS_{MM}$ is solute entropy. (delta sign means energy difference of complex and receptor plus ligand (complex – receptor – ligand))

Molecular mechanics energy change can be given as:

$$\Delta E_{MM} = \Delta E_{bonded} + \Delta E_{non-bonded} \quad (2)$$

$$\Delta E_{MM} = \Delta E_{bond} + \Delta E_{angle} + \Delta E_{dihed} + \Delta E_{improper} + \Delta E_{elec} + \Delta E_{vdW} \quad (3)$$

All the energetic terms present in equation (3) can be obtained from the molecular mechanical force field.

Free energy of solvation $\Delta G_{solv}$ has the contribution from electrostatic and non-electrostatic or non-polar interactions. Polar solvation free energy ($\Delta G_{pol,solv}$) is calculated from Poisson-Boltzmann (PB) equation and non-polar solvation free energy is calculated solvent accessible surface area term. For the calculation of entropic part normal mode or quasi harmonic analysis can be performed, and while comparing the binding free energies of multiple ligands this term can be dropped entirely. For the free energy calculation last 500 ns trajectories were taken for Apo-$SOD1^{SH}$-polyphenol complexes and the first 750 ns trajectories were taken for fibril-polyphenol complexes. We have neglected the entropic contribution to the free energy.

## Supplementary figures:

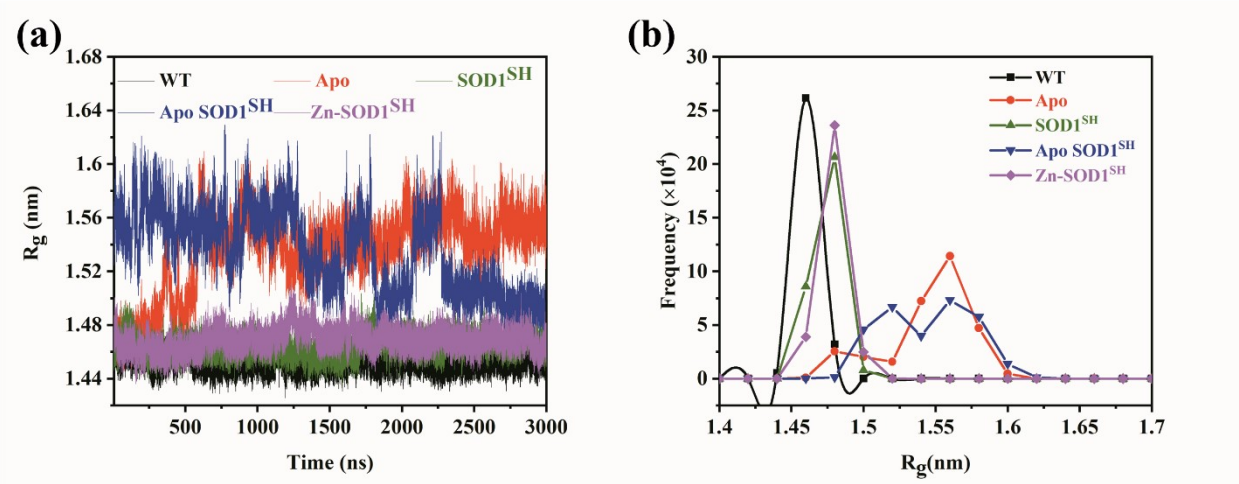



**Figure S1:** (a) Radius of gyration evolution plot of protein, (b) frequency distribution plot.

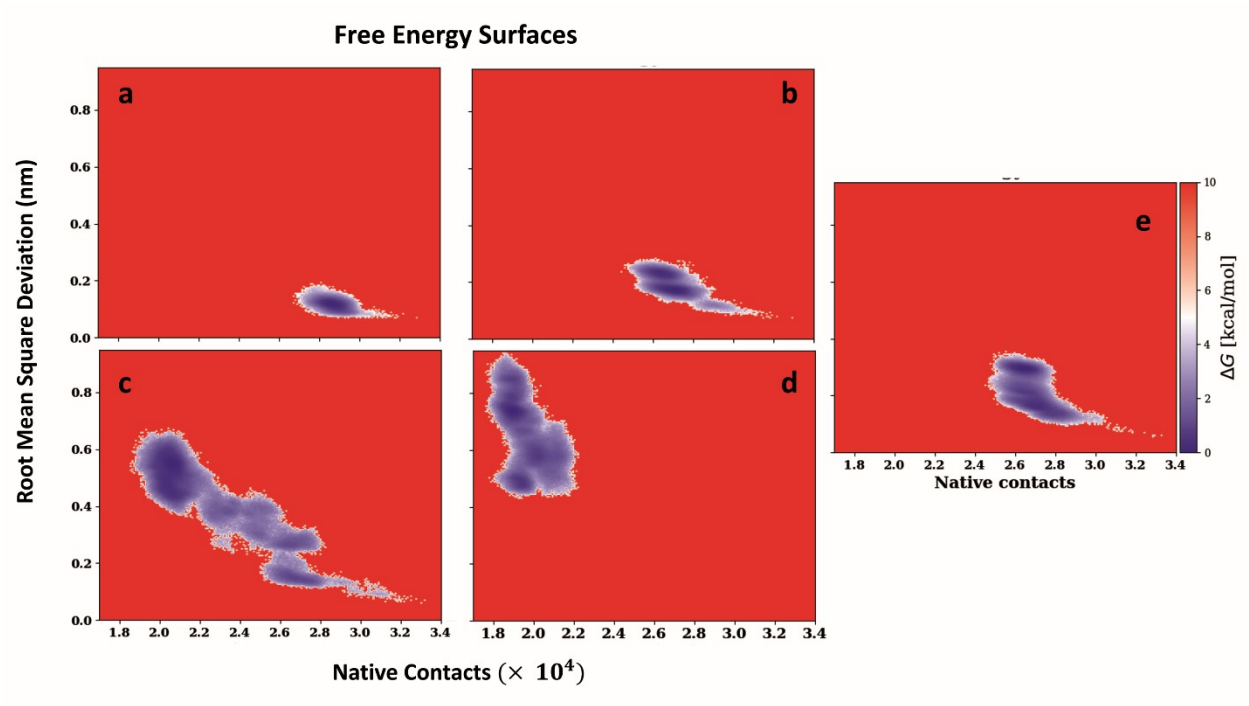



**Figure S2:** Free energy surface diagrams of (a) WT monomer, (b) SOD1$^{SH}$ monomer, (c) apo monomer, (d) apo SOD1$^{SH}$ monomer, and (e) Zn-SOD1$^{SH}$ for the complete trajectory, plotted as a function of RMSD and native contacts.

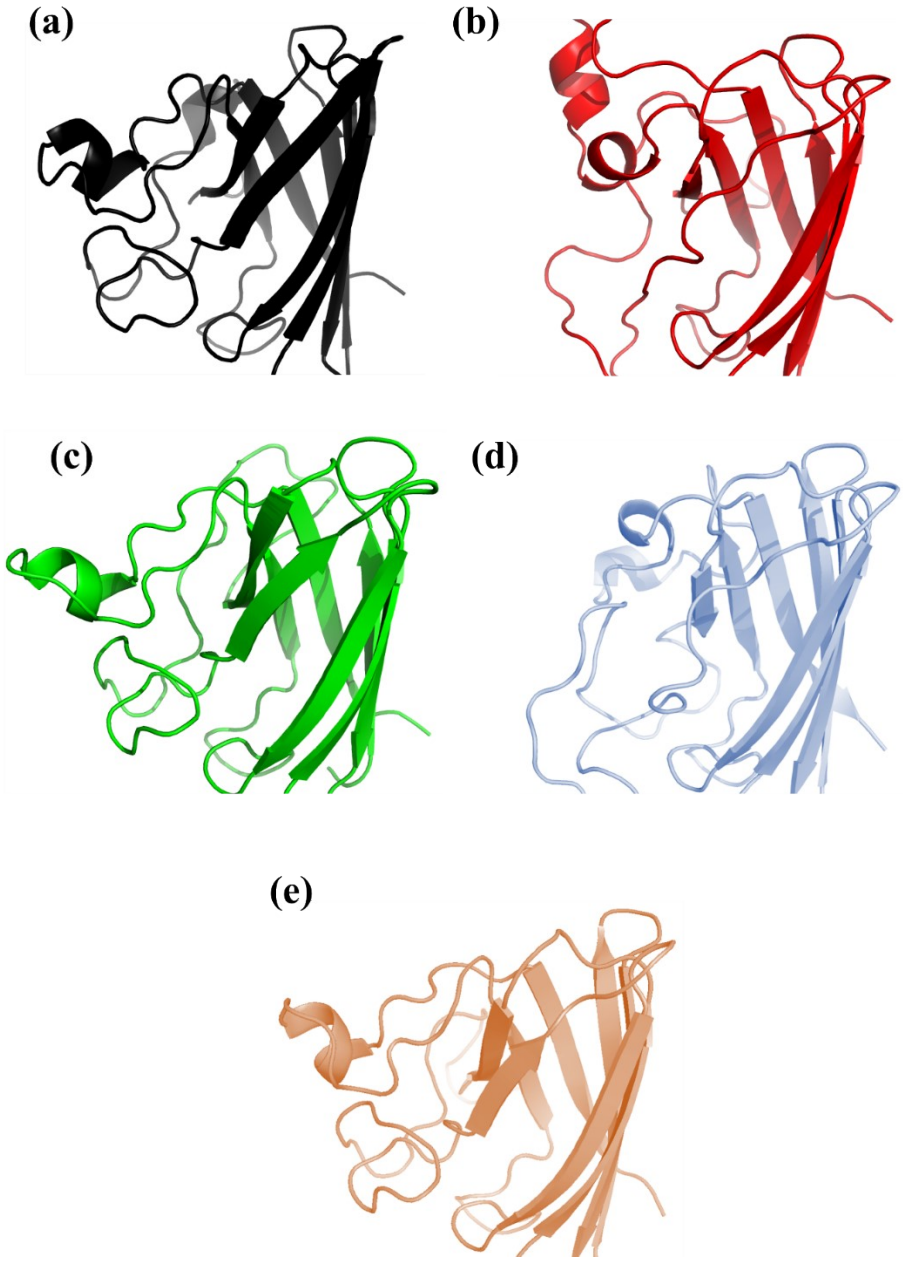



**Figure S3:** Pictorial representation of minimum energy conformations of (a) WT, (b) apo, (c) $SOD1^{SH}$, (d) apo-$SOD1^{SH}$ monomers, and (e) Zn-$SOD1^{SH}$ monomer obtained from free energy analysis.

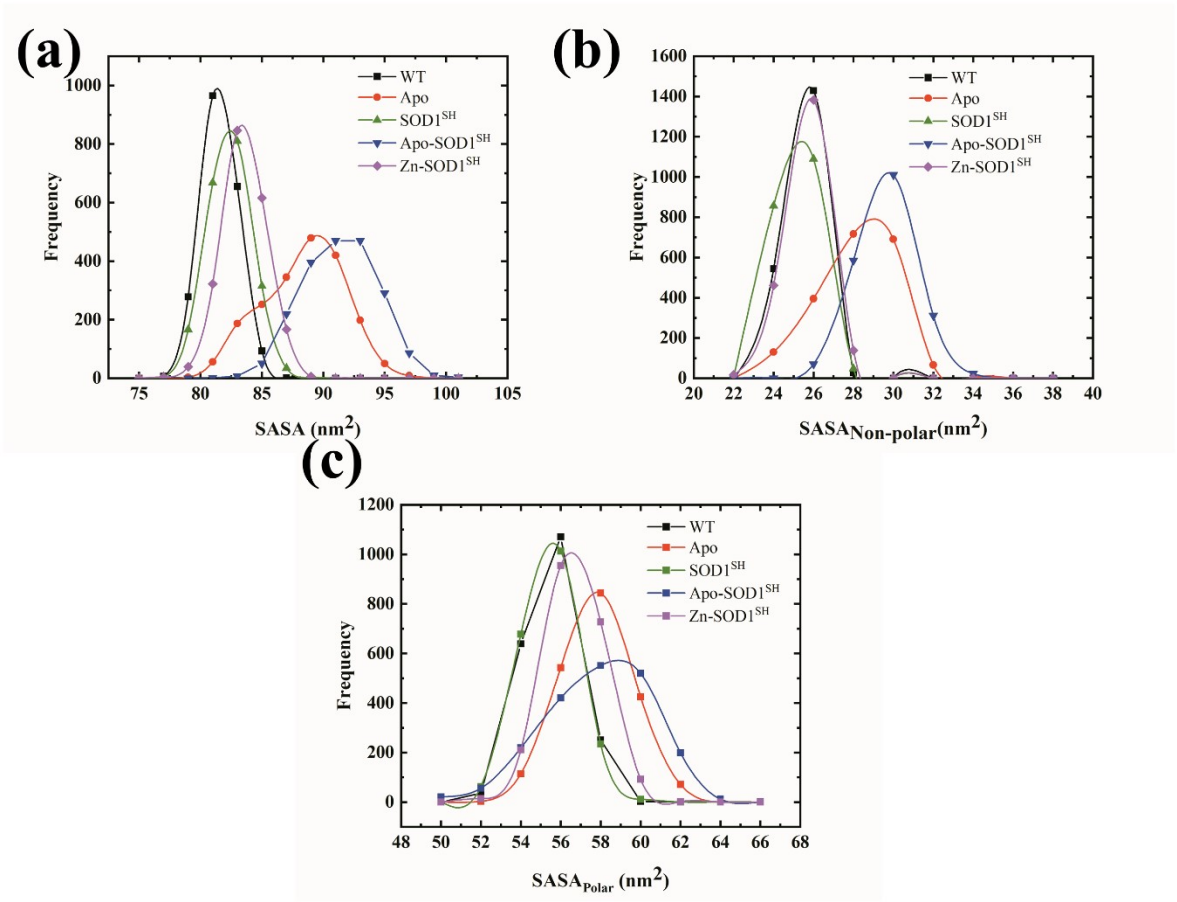



**Figure S4:** Solvent accessible surface area (SASA) plot for (a) all the residues, (b) polar residues, and (c) non-polar residues.

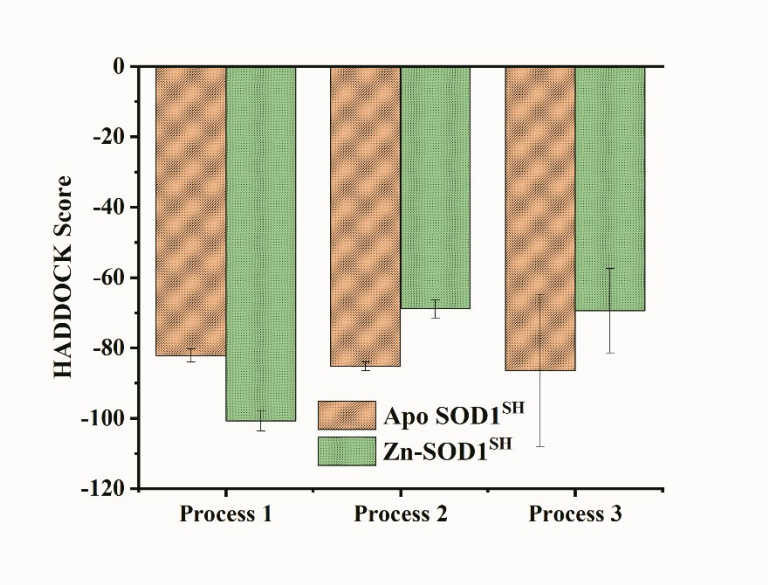



**Figure S5:** Protein-protein docking HADDOCK score obtained for the three dimer formation processes for Apo-SOD1$^{SH}$, and Zn-SOD1$^{SH}$ monomers.

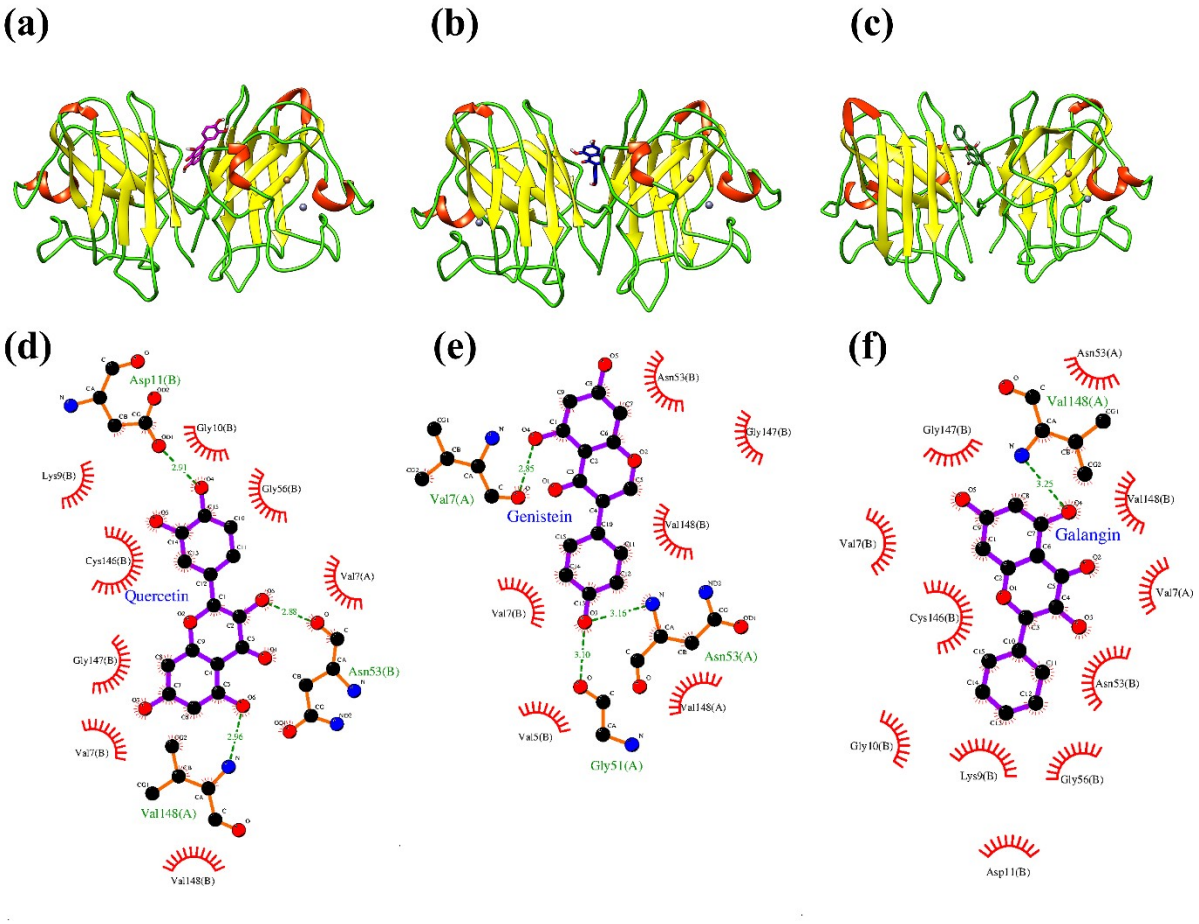



**Figure S6:** Visualization of interactions between WT SOD1 dimer and polyphenols (a) quercetin, (b) genistein and (c) galangin through LigPlot.

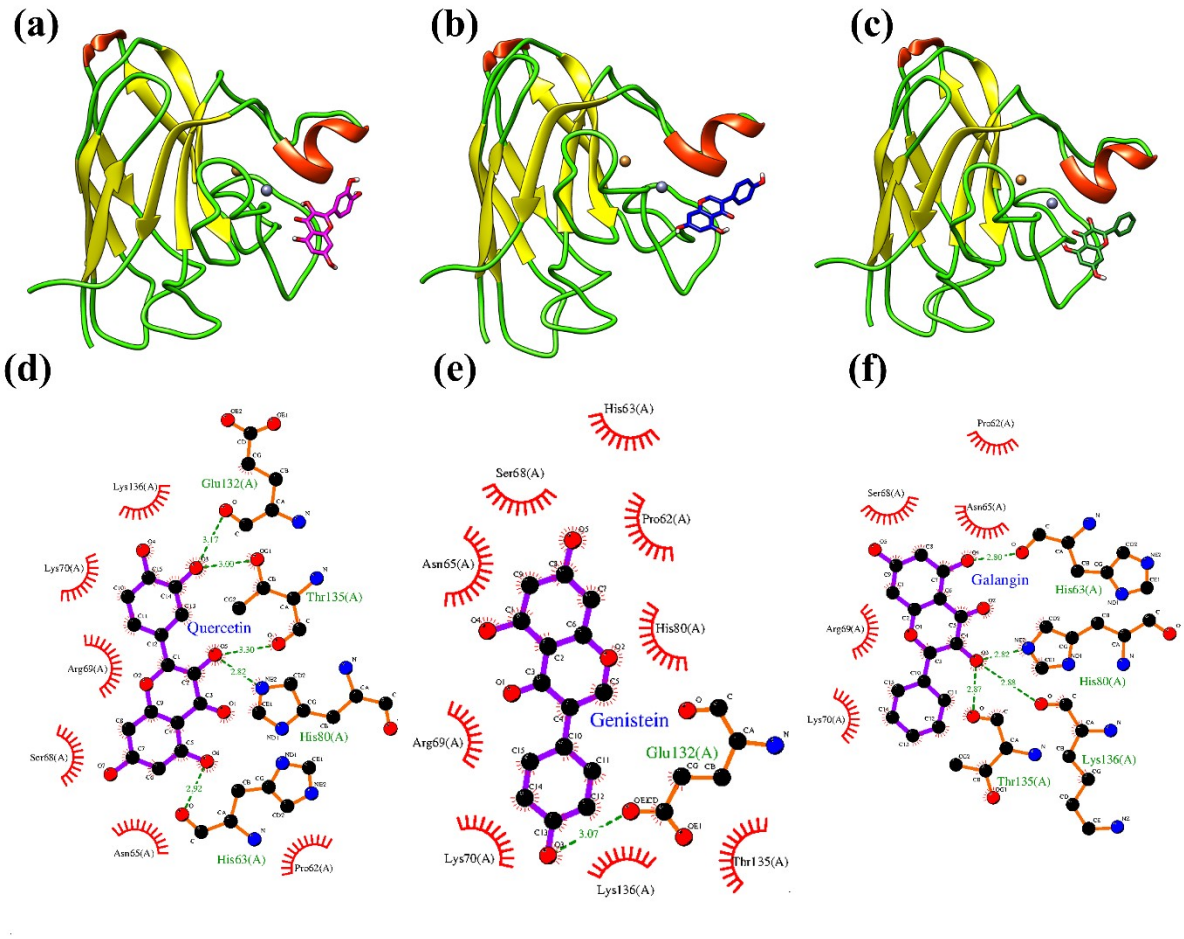



**Figure S7:** Visualization of interactions between WT SOD1 monomer and polyphenols (a) quercetin, (b) genistein and (c) galangin through LigPlot.

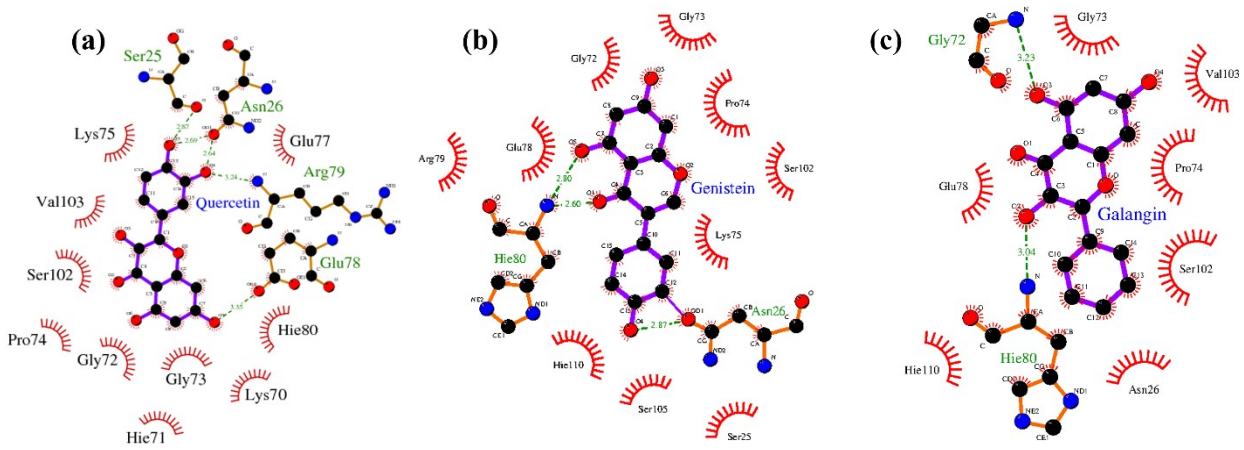



**Figure S8:** Visualization of interactions between apo-SOD1$^{SH}$ monomer and polyphenols (a) quercetin, (b) genistein and (c) galangin through LigPlot.

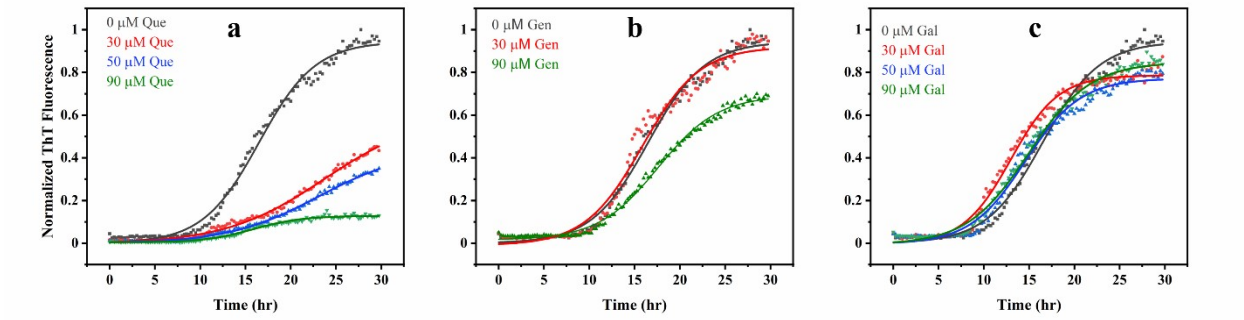



**Figure S9:** ThT aggregation kinetics of SOD1 protein in the absence and presence of different concentrations of (a) quercetin, (b) genistein, and (c) galangin.

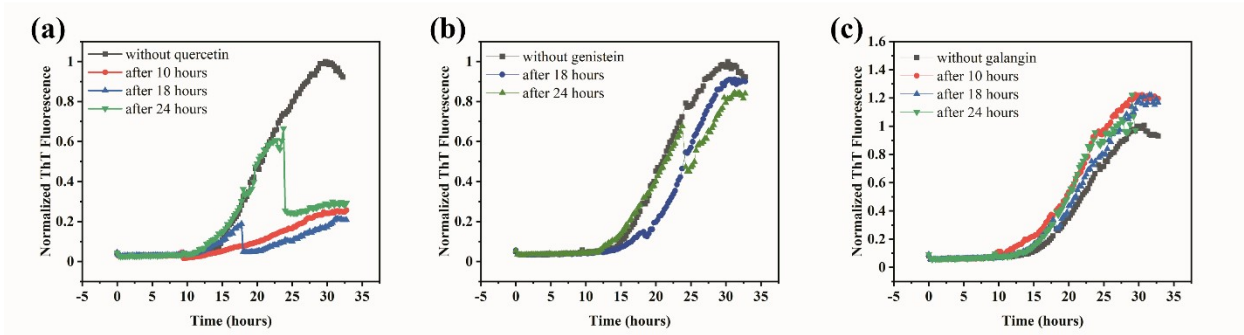


**Figure S10:** ThT aggregation kinetics of SOD1 in the presence of 90 µM polyphenol (a) quercetin, (b) genistein, and (c) galangin added at different time points at 10 hour, 18 hour and 24 hour.

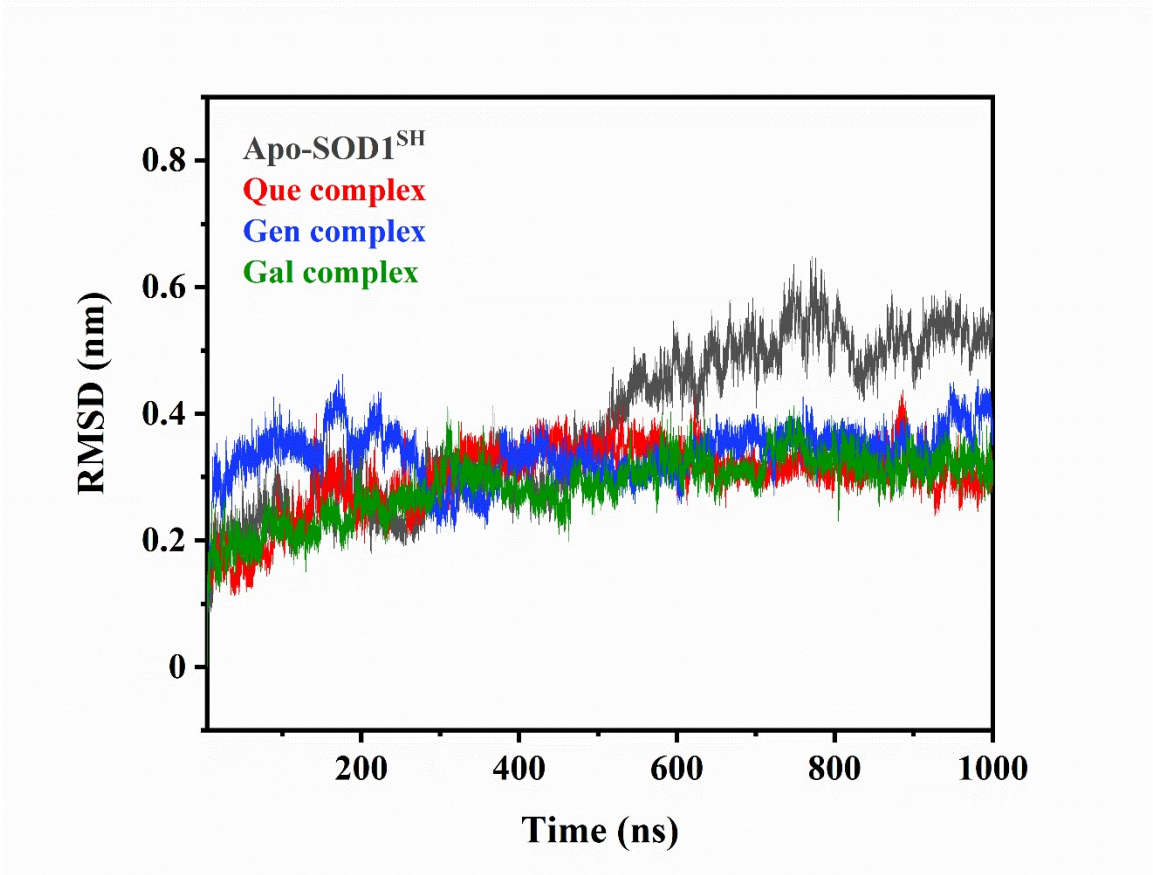



**Figure S11:** Cα RMSD plot for apo-SOD1$^{SH}$ monomer alone and in complexed state.

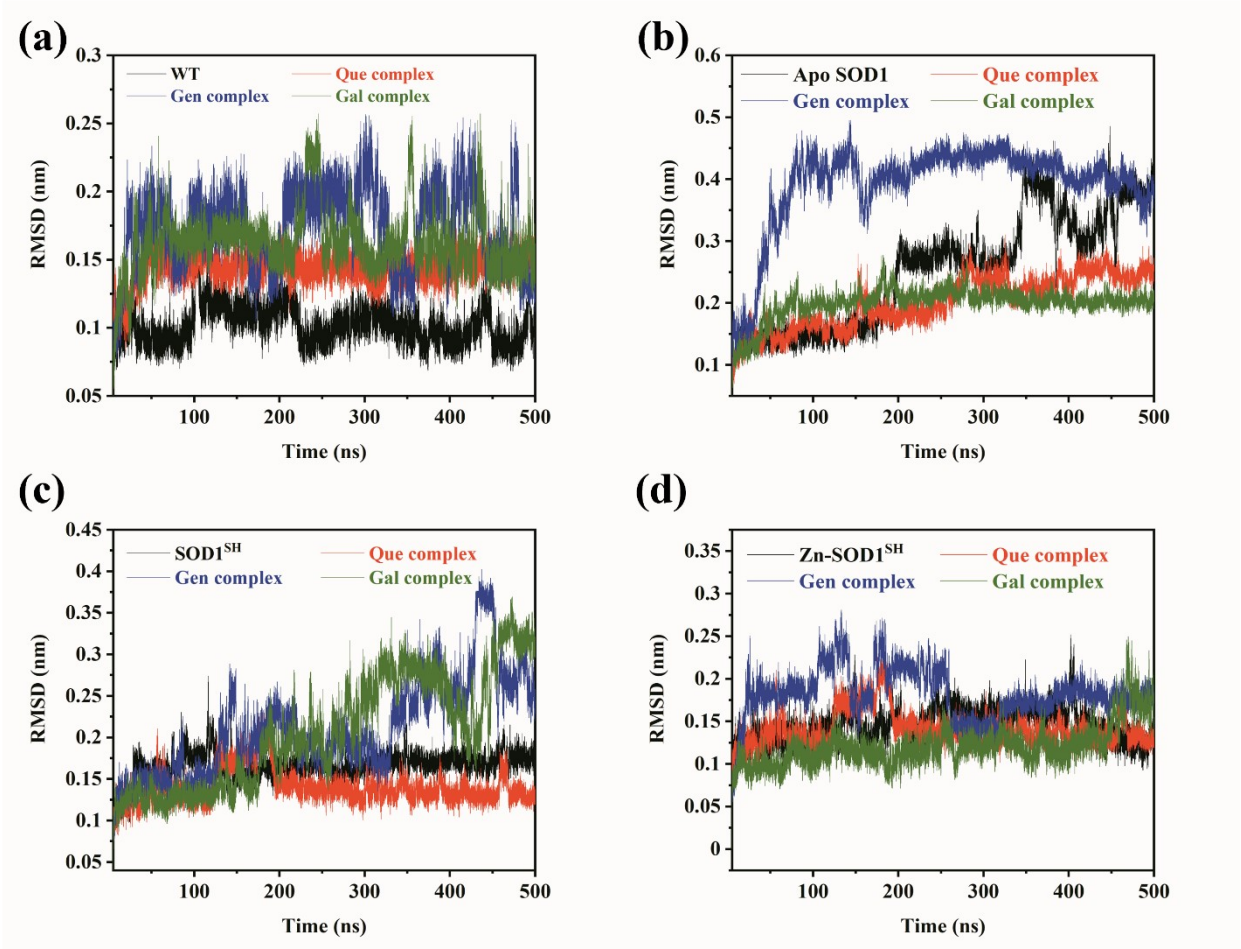



**Figure S12:** Cα RMSD plot for (a) SOD1 monomer alone, (b) apo monomer alone, (c) $SOD1^{SH}$ alone and (d) Zn-$SOD1^{SH}$ alone and in complexed state.

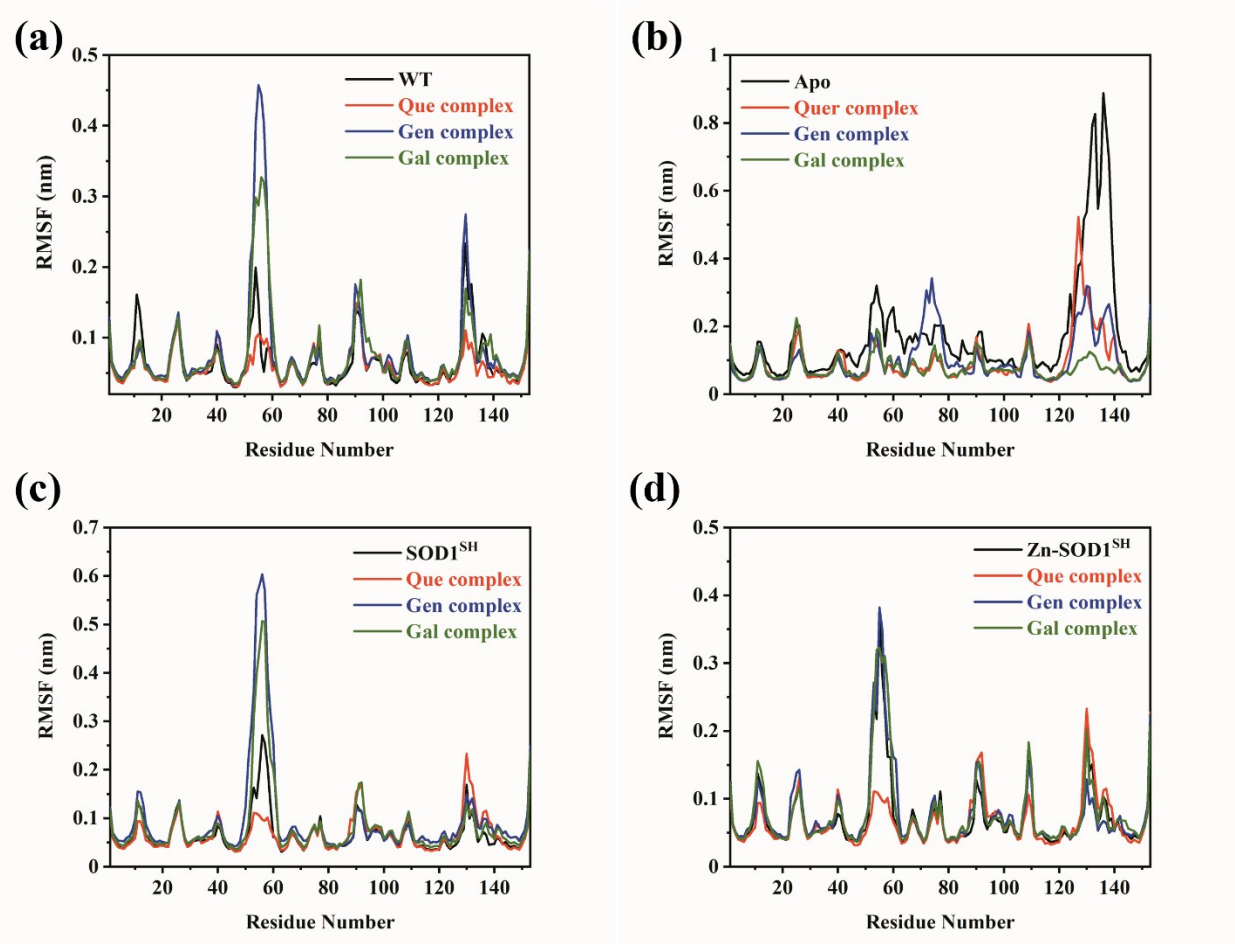



**Figure S13:** Cα RMSF plot for (a) SOD1 monomer alone, (b) apo monomer alone, (c) $SOD1^{SH}$ alone and (d) Zn-$SOD1^{SH}$ alone and in complexed state, for the last 200 ns.

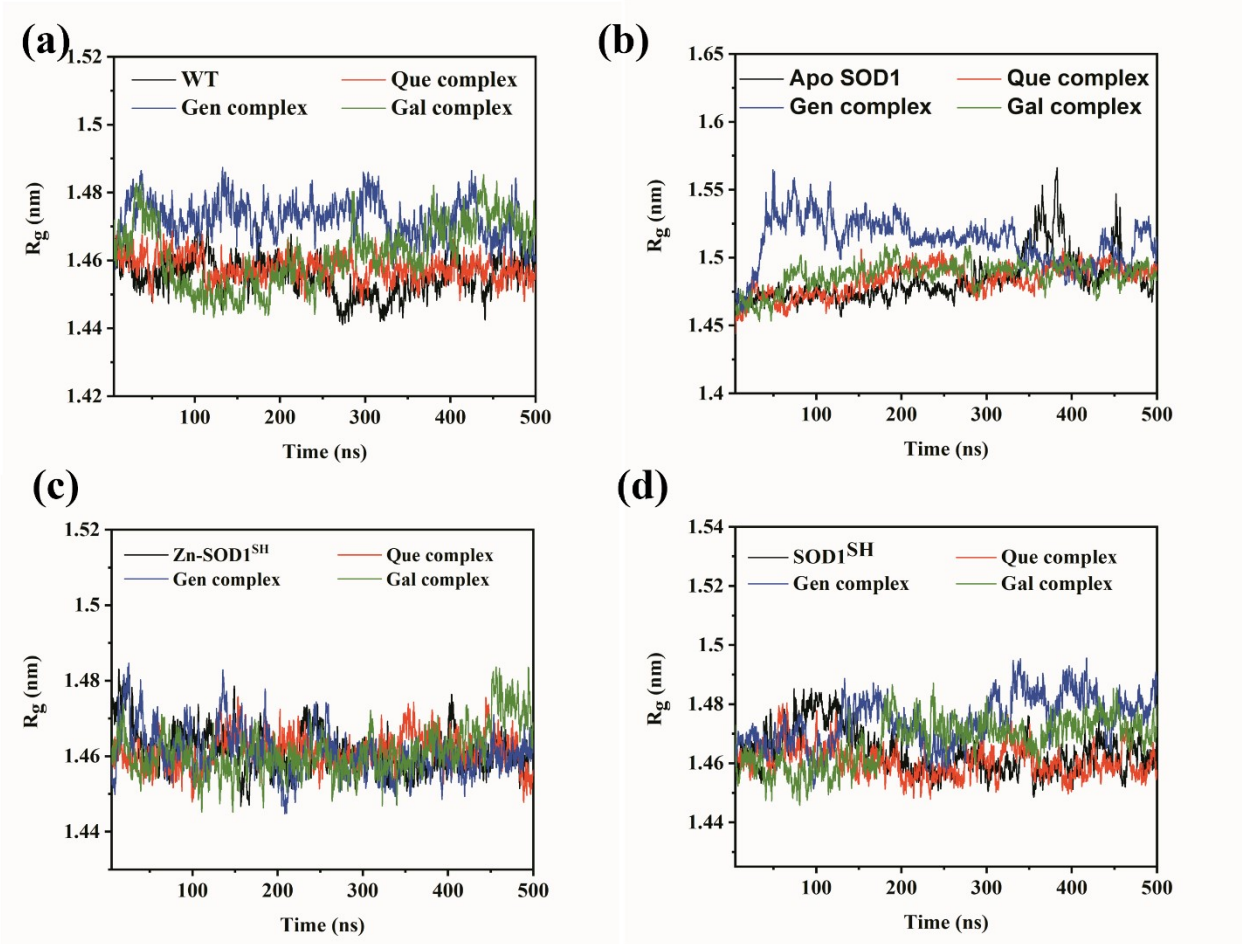



**Figure S14:** All atom radius of gyration ($R_g$) plot for (a) SOD1 monomer alone, (b) apo monomer alone, (c) $SOD1^{SH}$ alone and (d) Zn-$SOD1^{SH}$ alone and in complexed state.

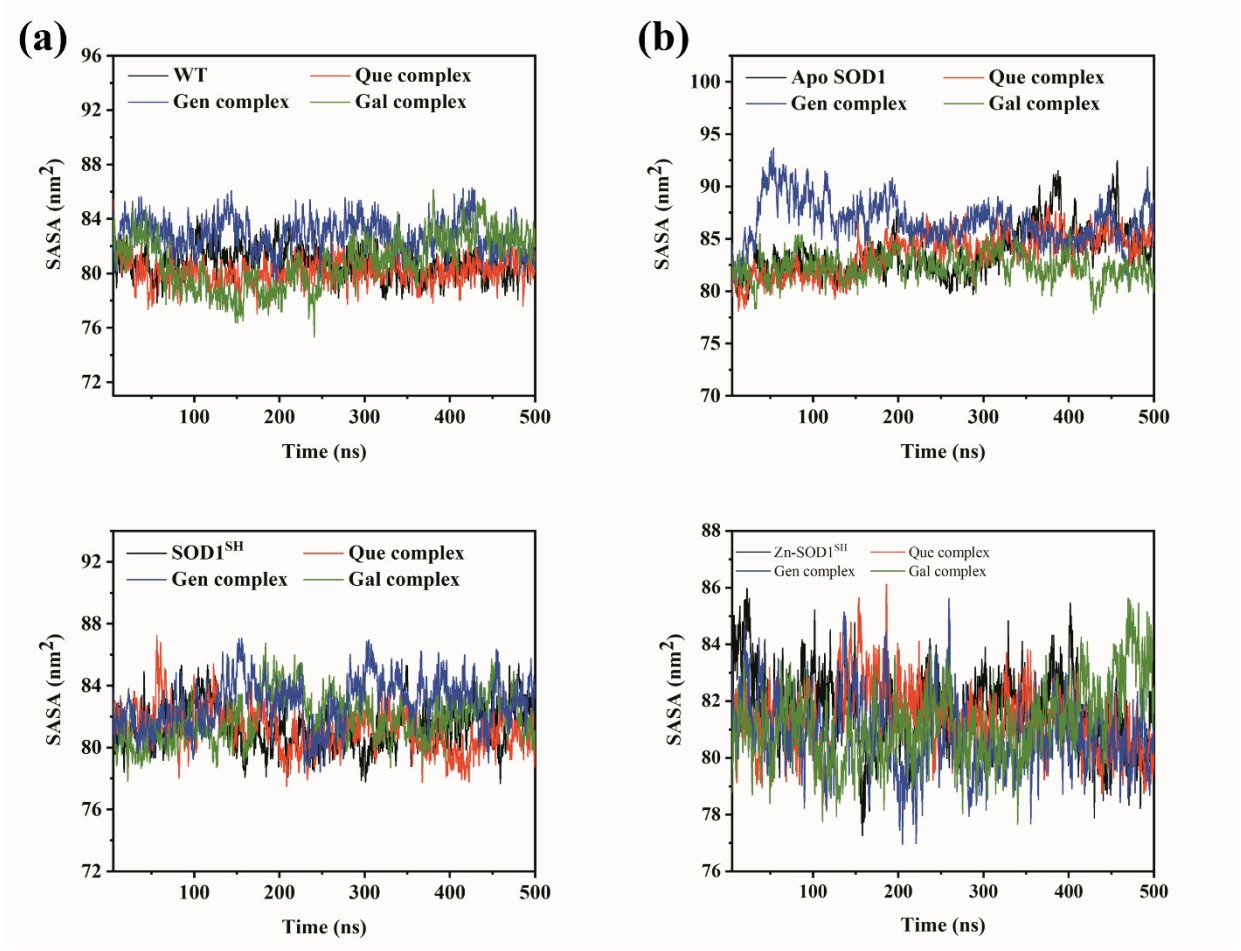



**Figure S15:** All atom solvent accessible surface area (SASA) plot for (a) SOD1 monomer alone, (b) apo monomer alone, (c) $SOD1^{SH}$ alone and (d) Zn-$SOD1^{SH}$ alone and in complexed state.

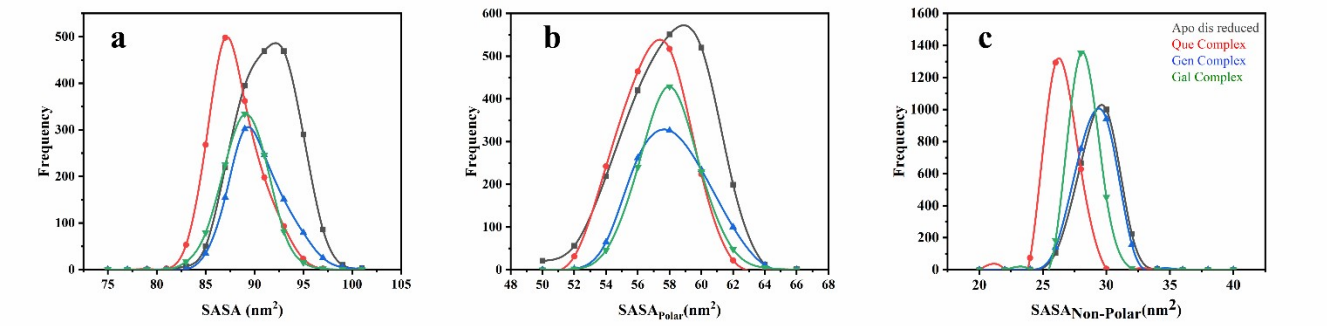


**Figure S16:** Probability distribution of SASA values for (a) total residues, (b) polar residues, and (c) Non-polar residues of apo-SOD1$^{SH}$ monomer, and polyphenol complexes.

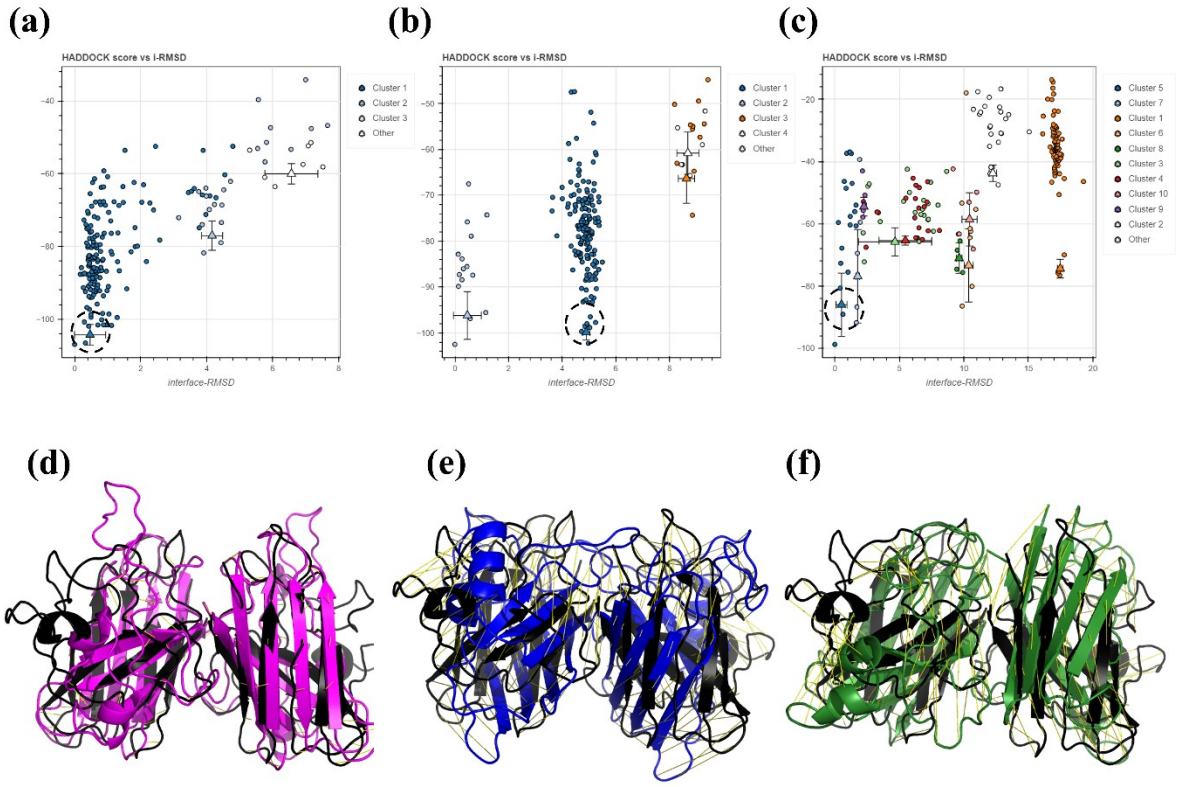



**Figure S17:** HADDOCK score versus interface-RMSD for (a) quercetin, (b) genistein, and (c) galangin bound apo-SOD1$^{SH}$ homodimer formation through process 1. The average value of HADDOCK score was calculated by taking the average of best 4 structures of each cluster. The cluster averages and standard deviations are indicated by colored dots with associated error bars.

The cluster chosen as the best representation of dimer is highlighted by dotted circle and the structure obtained from that cluster is used for the comparison with WT dimer crystal structure (PDB: 1SPD). Comparison of structure of dimer obtained from the protein-protein docking of polyphenol complexes (d) quercetin, (e) genistein, and (f) galangin with the structure of WT homodimer.

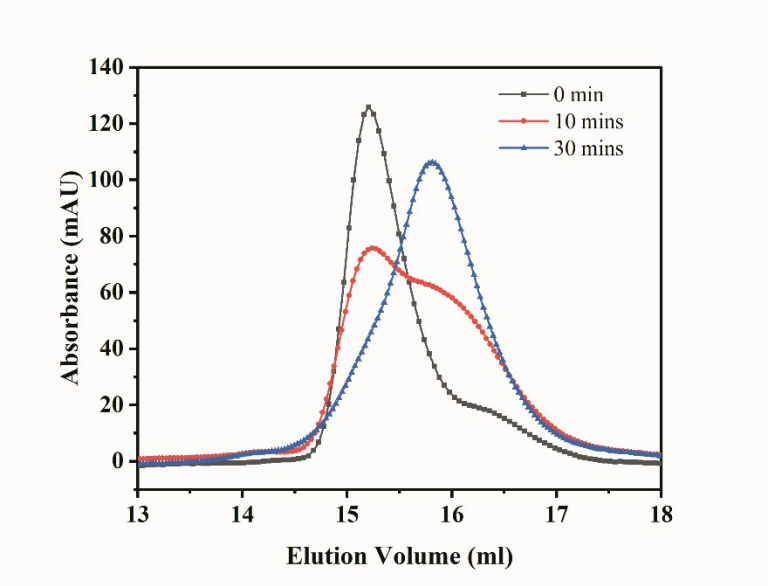



**Figure S18:** Size exclusion chromatogram of EDTA and DTT-treated SOD1 at different times.

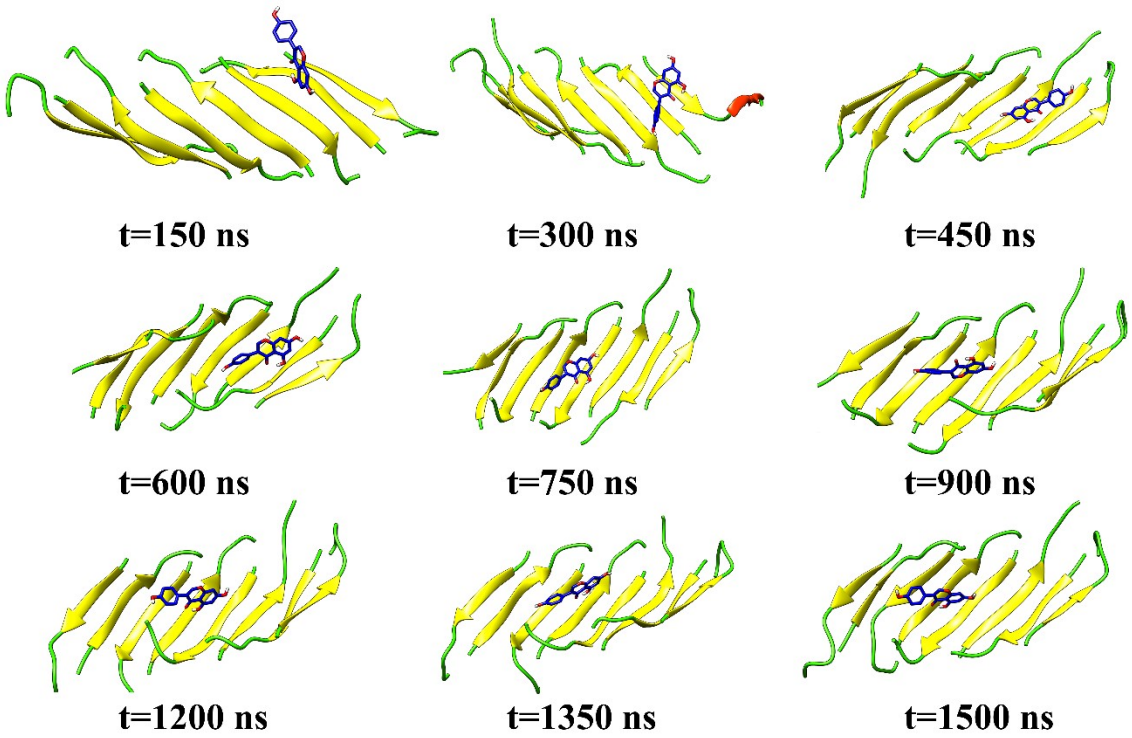



**Figure S19:** Visualization of frames extracted at different time points from the fibril-genistein MD simulation trajectory.

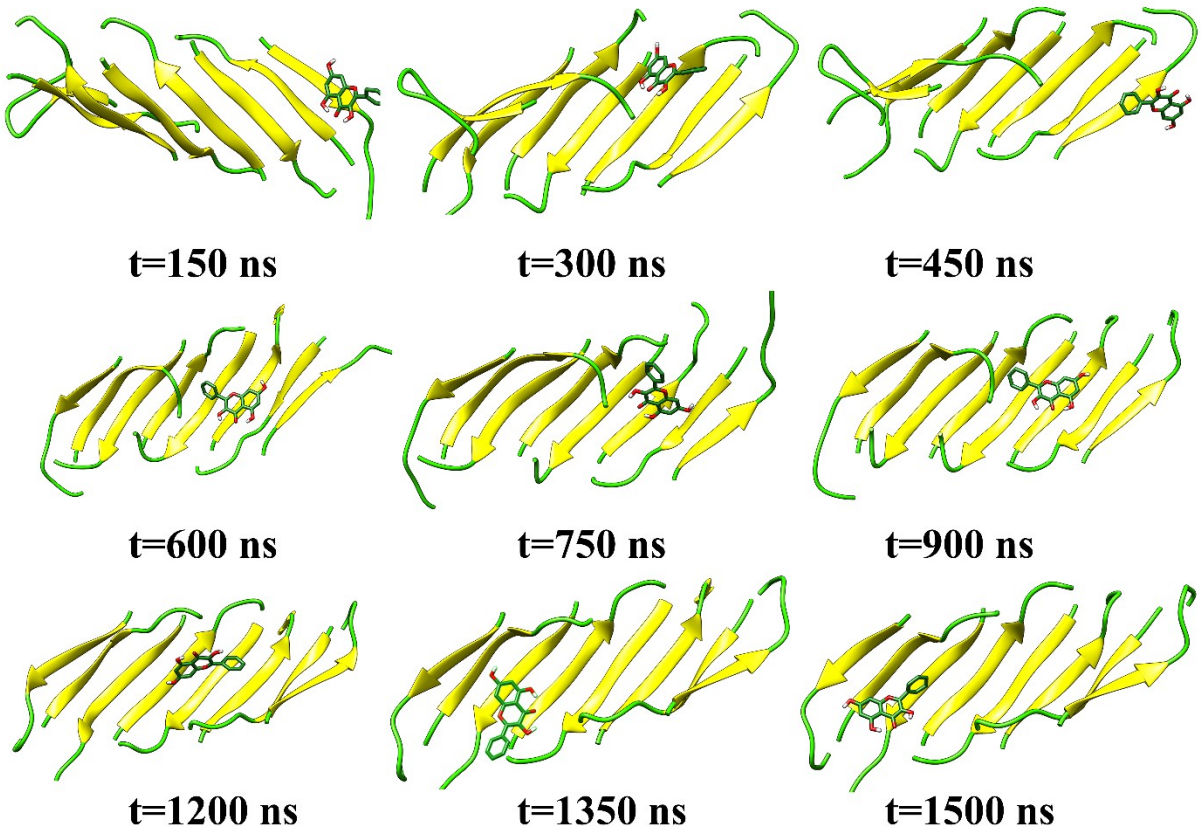



**Figure S20:** Visualization of frames extracted at different time points from the fibril-galangin MD simulation trajectory.

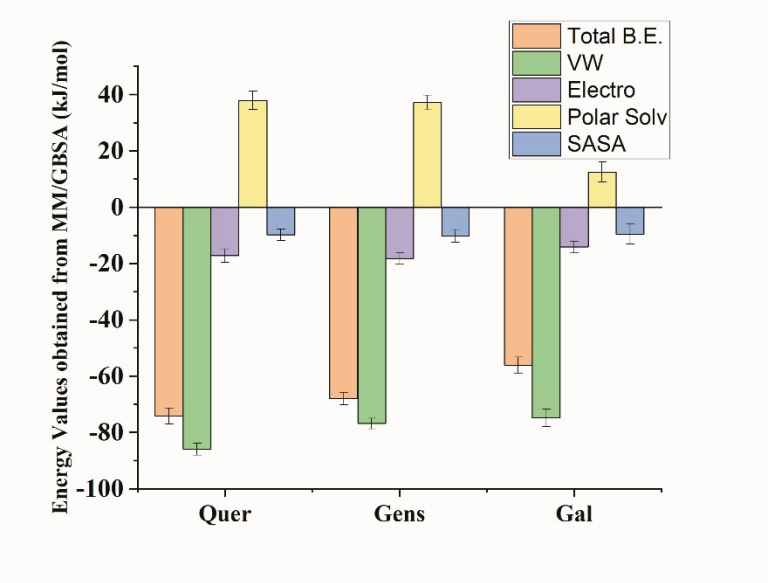



**Figure S21:** MM/GBSA results obtained for the polyphenols binding to the SOD1 fibril for the 750 ns trajectory.

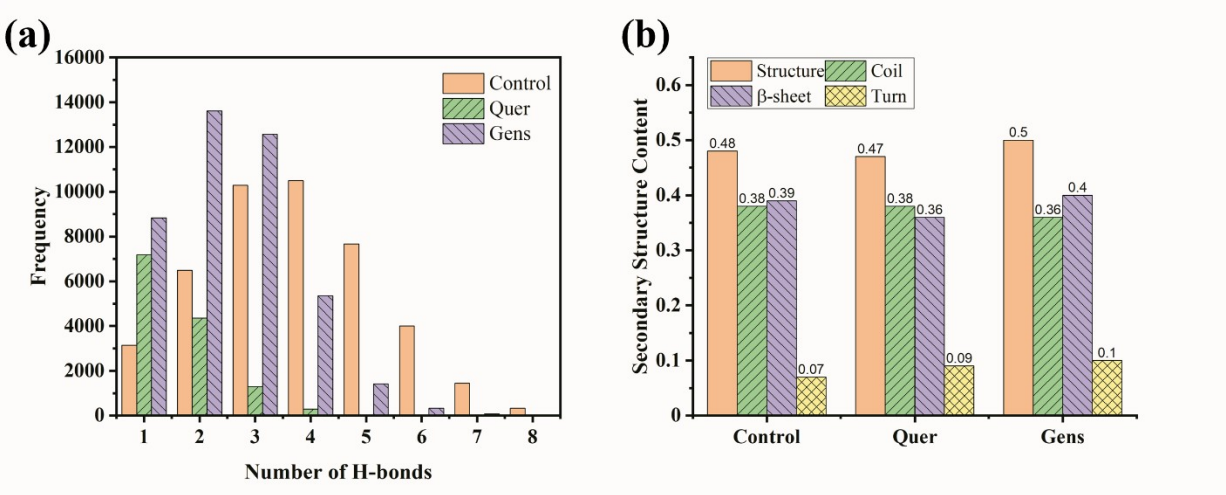



**Figure S22: (a)** The probability distribution of hydrogen bonds formed between existing octamer fibril and added monomer in the absence and presence of quercetin and genistein, (b) secondary structure content in the elongating fibril in the absence and presence of polyphenols.

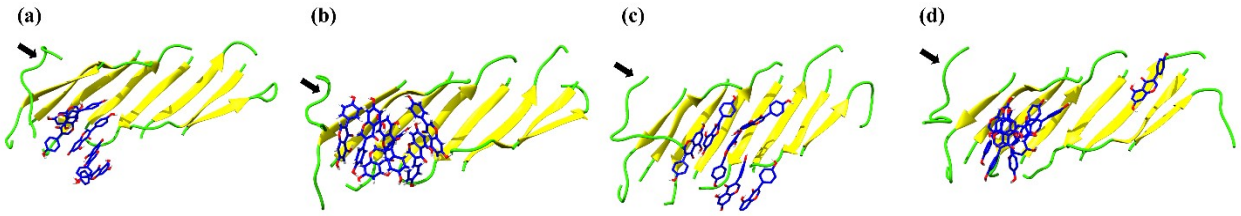



**Figure S23:** Snapshots obtained from RMSD based clustering analysis the simulation of (a) 0-100 ns, (b) 100-200 ns, (c) 200-400 ns, and (d) 400-500 ns trajectory of SOD1 fibril elongation in the presence of genistein. The black arrow indicates the position of added SOD1 monomeric peptide.

**Supplementary table**

**Table S1:** List of exposed residues upon erroneous PTMs and their location in protein structure.

| S.No. | System | Exposed residues | Protein structure |
|---|---|---|---|
| 1 | Apo | 7,40,42,49,52,53,56,60,63,70-71,74,76,79,80,83,86,90,101,124,133,135-140 | β1, L3, L4, β5, L5, β6, L7 |
| 2 | $SOD1^{SH}$ | 49,53,56-57,69,77,96,115,122,130,137 | L4, β6, L7 |
| 3 | Zn-$SOD1^{SH}$ | 24,40,53,56,61,69,74,77,90-91,130,137,143,146 | L2, L3, L4, L5, L7, β8 |
| 4 | Apo-$SOD1^{SH}$ | 3,40,42,45,52,56-57,60,63,64,66,71,72,74,82-86,90,124,131-132,134-135,137-138,141-142 | β1, L3, β4, L4, β5, L5, L7 |

**Table S2.** HADDOCK results for guided docking of protein.

| SOD1 variants | Process 1 | | | | Process 3 | | | | Process 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HD Score | vdW | Electrostatic | Desol | HD Score | vdW | Electrostatic | Desol | HD Score | vdW | Electrostatic | Desol |
| WT | -84.7 ± 2.4 | -47.2 ± 3.4 | -164.1 ± 28.3 | -7 ± 1.1 | -72.9 ± 3 | -49.8 ± 3.8 | -62.6 ± 15.2 | -11.5 ± 1.3 | -63.7 ± 5 | -44 ± 5.8 | -102.7 ± 45.7 | -13.4 ± 4.2 |
| Apo | -73.6 ± 8.1 | -34.9 ± 3.9 | -257.1 ± 11.1 | 4.8 ± 4.4 | -66.9 ± 4.7 | -32.6 ± 1.8 | -237.4 ± 24.1 | 10.9 ± 1.6 | -85.3 ± 4.9 | -48.6 ± 3.1 | -273 ± 16.7 | -5.8 ± 1.0 |
| $SOD1^{SH}$ | -94 ±2.7 | -61.5 ± 6.8 | -112.5 ± 47.7 | -17.7 ± 4.5 | -92.9 ± 9.1 | -56.5 ± 2.5 | -132.2 ± 21.1 | -13.2 ± 3.2 | -91 ± 7.8 | -60.7 ± 3.7 | -153.6 ± 25.7 | -12.9 ± 2.1 |
| Apo-$SOD1^{SH}$ | -82.1 ± 1.9 | -61.1 ± 3.8 | -40.6 ± 22.2 | -17.1 ± 3.5 | -85.2 ± 1.2 | -60.5 ± 3 | -110.2 ± 30.1 | -7.8 ± 4.1 | -86.4 ± 21.6 | -52.6 ± 6.7 | -223 ± 80.7 | -4.5 ± 1.8 |
| Zn-$SOD1^{SH}$ | -100.7 ± 2.9 | -68.3 ± 1 | -190.6 ± 12.2 | -3.6 ± 2.1 | -68.9 ± 2.6 | -65.4 ± 2.5 | -15.7 ±6.7 | -1.4 ± 1.9 | -69.4 ± 12 | -48.2 ± 9 | -151.5 ± 28.1 | -0.2 ± 1.7 |

**Table S3.** List of polyphenols with their binding energies and binding residues obtained from blind docking with WT, apo, $SOD1^{SH}$, Zn-$SOD1^{SH}$ and apo-$SOD1^{SH}$ monomers.

| S.No. | Target | Polyphenols | Binding Energy (kCal/mol) | Binding residues |
|---|---|---|---|---|
| 1 | WT Dimer | Quercetin | -7.7 | Val 7(A), Val 148(A), Val 7(B), Lys 9(B), Gly 10(B), Asp 11(B), Asn 53(B), Gly 56(B), Cys 146(B), Gly 147(B) |
| | | Genistein | -7.0 | Val 7(A), Gly 51(A), Asn 53(A), Val 5(B), Val 7(B), Asn |

| | | | | |
|---|---|---|---|---|
| | | | | 53(B), Gly 147(B), Val 148(B) |
| | | Galangin | -7.5 | Val 7(A), Asn 53(A), Val 148(A), Val 7(B), Lys 9(B), Gly 10(B), Asp 11(B), Asn 53(B), Gly 56(B), Cys 146(B), Gly 147(B), Val 148(B) |
| 2 | WT Monomer | Quercetin | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135, Lys 136 |
| | | Genistein | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135, Lys 136 |
| | | Galangin | -7.0 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Thr 135, Lys 136 |
| 3 | Apo | Quercetin | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135, Lys 136 |
| | | Genistein | -6.8 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135, Lys 136 |
| | | Galangin | -7.0 | Pro 66, Leu 67, Arg 69, Glu 77, Arg 79, His 80, Val 81, Val 103 |

| | | | | |
|---|---|---|---|---|
| 4 | $SOD1^{SH}$ | Quercetin | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135 |
| | | Genistein | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135, Lys 136 |
| | | Galangin | -7.1 | Pro 66, Leu 67, Arg 69, Glu 77, Arg 79, His 80, Val 81 |
| 5 | $Zn\text{-}SOD1^{SH}$ | Quercetin | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Thr 132, Thr 135 |
| | | Genistein | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Glu 132, Thr 135, Lys 136 |
| | | Galangin | -6.9 | Pro 62, His 63, Asn 65, Ser 68, Arg 69, Lys 70, His 80, Thr 135, Lys 136 |
| 6 | $Apo\text{-}SOD1^{SH}$ | Quercetin | -7.83 | Ser 25, Asn 26, Lys 70, His 71, Gly 72-73, Pro 74, Lys 75, His 80, Ser 102, Val 103 |
| | | Genistein | -8.1 | Ser 25, Asn 26, Gly 72, Gly 73, Pro 74, Lys 75, Glu 78, Arg 79, His 80, Ser 105, His 110 |

| | | | | |
|---|---|---|---|---|
| | | Galangin | -7.9 | Asn 26, Gly 72, Gly 73, Pro 74, Glu 78, His 80, Ser 102, Val 103 |

**Table S4:** List of polyphenols with their binding energies and binding residues obtained from blind docking with 11 residue octameric corkscrew fibril.

| S.No. | Polyphenols | Binding Energy (kCal/mol) | Binding residues |
|---|---|---|---|
| 1 | Quercetin | -6.48 | Trp 32(A), Lys 30(B), Val 31(B), Trp 32(B), Gly 33(C) |
| 2 | Genistein | -5.93 | Lys 28(B), Lys 30(B), Val 31(B), Trp 32(A), Trp 32(B), Trp 32(C), Gly 33(C) |
| 3 | Galangin | -6.38 | Lys 30(B), Val 31(B), Trp 32(A), Trp 32(B), Gly 33(C) |

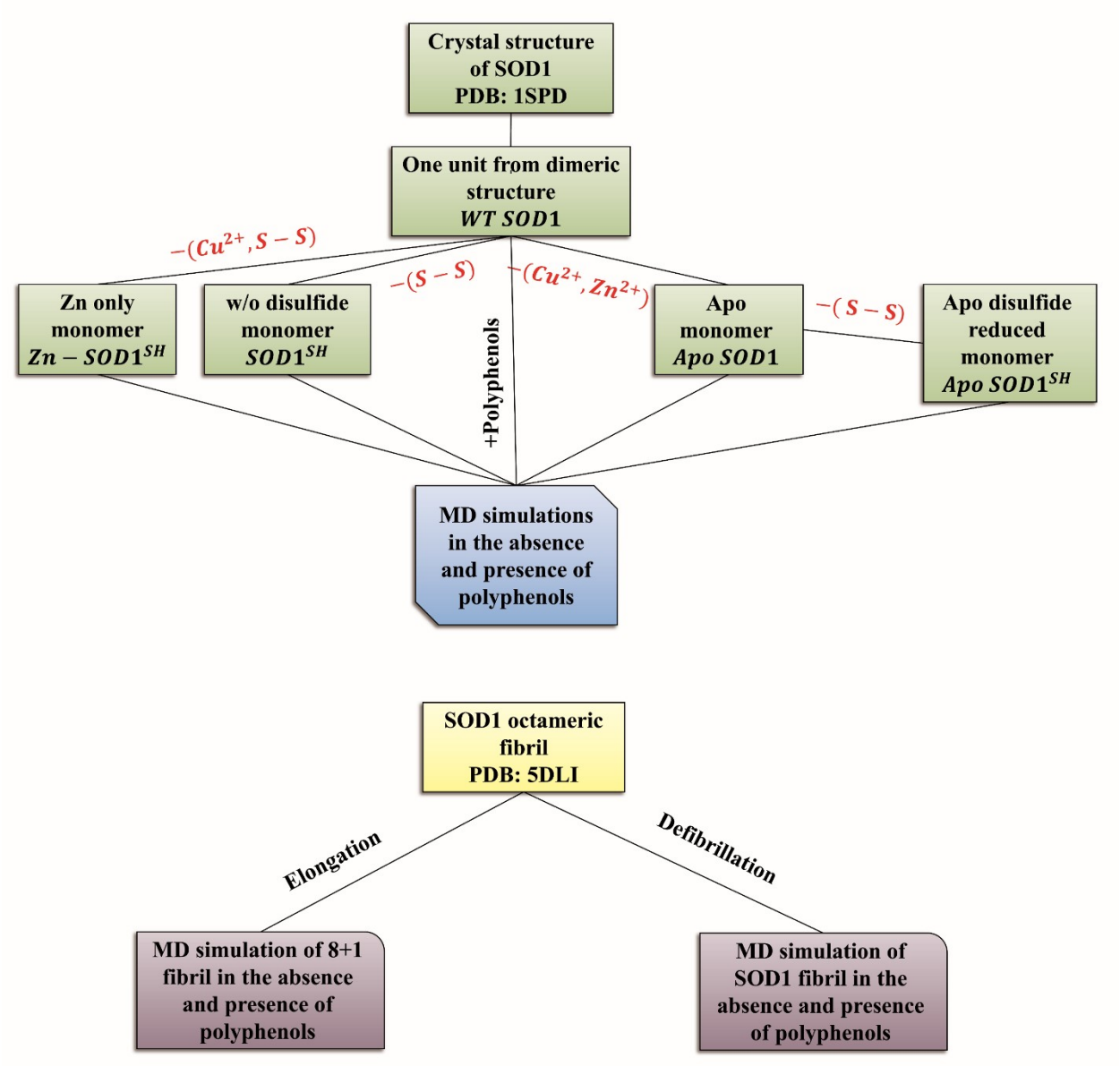
**Table S5:** Binding energies of Apo-SOD1$^{SH}$/SOD1 fibril-polyphenol complexes obtained from MM/PBSA approach by taking the last 500 ns trajectories.

| S.No. | Target | Ligand | Binding Energy (kJ/mol) |
|---|---|---|---|
| 1 | Apo-SOD1$^{SH}$ | Quercetin | -64.96 ± 1.56 |
| | | Genistein | -47.76 ± 1.50 |

| | | | |
|---|---|---|---|
| | | Galangin | -32.39 ± 3.10 |
| 2 | 5DLI SOD1 fibril | Quercetin | -74.97 ± 2.80 |
| | | Genistein | -68.021 +/- 2.21 |
| | | Galangin | -56.115 +/- 2.85 |

**Supplementary Scheme**

**Scheme S1:** A flow chart summarizing the system preparation process for all the simulations.


Crystal structure of SOD1 PDB: 1SPD
One unit from dimeric structure WT SOD1
$-(Cu^{2+}, S-S)$
$-(S-S)$
$-(Cu^{2+}, Zn^{2+})$
$-(S-S)$
Zn only monomer $Zn-SOD1^{SH}$
w/o disulfide monomer $SOD1^{SH}$
+Polyphenols
Apo monomer Apo SOD1
Apo disulfide reduced monomer $Apo\ SOD1^{SH}$
MD simulations in the absence and presence of polyphenols
SOD1 octameric fibril PDB: 5DLI
Elongation
Defibrillation
MD simulation of 8+1 fibril in the absence and presence of polyphenols
MD simulation of SOD1 fibril in the absence and presence of polyphenols

**Scheme S2:** Diagrammatical representation of protein-protein docking process through HADDOCK.