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## 1 Results and Discussion

Our comprehensive study on the wild type and variants demonstrates the evolution of glutathione peroxidase protein 6 cys and selenium protein. Despite the differences in the cysteine and selenocysteine parameters in the system, they show a wide range of associated evolutionary pathways. We first optimized the reaction, one step in a stepwise mechanism going from selenol to selenolate of selenocysteine and from thiol to thiolate ion in the case of cysteine, while setting up the Empirical Valence Bond Simulation. The substrate hydrogen peroxide was placed at the distance from selenocysteine/cysteine and glutamine83 as mentioned in the QM/MM calculations by Morokuma.

### 1.1 Free Energy Changes

Here, we present the free energy changes for the wild type and mutant systems. The table below summarizes the mean free energy values (Mean  $dG^*$  and Mean  $dG_0$ ) for the systems we analyzed.

All the systems listed have positive  $dG^*$  values, indicating that the processes are non-spontaneous under the experimental conditions. All systems exhibit negative  $dG_0$  values, suggesting that they are spontaneous under standard conditions. The variability in  $dG_0$  values among the mutant systems suggests that the specific mutations (e.g., S47A, F48Y) can significantly influence the free energy changes, potentially reflecting changes in stability or interactions within the system.

| System                               | Mean dG* (kcal/mol)       | Mean dG0 (kcal/mol)        |
|--------------------------------------|---------------------------|----------------------------|
| WT-Mouse Cys                         | 16.45 $\pm$ 0.56 kcal/mol | -59.70 $\pm$ 1.54 kcal/mol |
| WT-Human Sec                         | 13.69 $\pm$ 0.95 kcal/mol | -64.82 $\pm$ 1.69 kcal/mol |
| S47A,F48Y,T54Q,R99C                  | 19.34 $\pm$ 0.58 kcal/mol | -47.63 $\pm$ 2.85 kcal/mol |
| F48Y,T52A,T54Q,R99C                  | 19.83 $\pm$ 0.72 kcal/mol | -39.63 $\pm$ 2.80 kcal/mol |
| S47A,F48Y,T52A,R99C                  | 18.03 $\pm$ 0.62 kcal/mol | -52.45 $\pm$ 2.62 kcal/mol |
| S47A,T52A,T54Q,R99C                  | 14.40 $\pm$ 0.80 kcal/mol | -61.56 $\pm$ 2.70 kcal/mol |
| S47A,F48Y,T52A,T54Q                  | 17.78 $\pm$ 0.89 kcal/mol | -57.35 $\pm$ 2.19 kcal/mol |
| Mouse Cys - S47A,F48Y,T52A,T54Q,R99C | 16.27 $\pm$ 0.50 kcal/mol | -62.54 $\pm$ 1.60 kcal/mol |
| Mouse Sec - S47A,F48Y,T52A,T54Q,R99C | 12.21 $\pm$ 0.62 kcal/mol | -69.17 $\pm$ 1.64 kcal/mol |
| Human Cys - A47S,Y48F,A52T,Q54T,C99R | 12.31 $\pm$ 0.51 kcal/mol | -52.07 $\pm$ 3.12 kcal/mol |
| S47A                                 | 14.36 $\pm$ 0.51 kcal/mol | -60.44 $\pm$ 2.41 kcal/mol |
| F48Y                                 | 16.91 $\pm$ 0.42 kcal/mol | -55.41 $\pm$ 1.82 kcal/mol |
| T52A                                 | 15.84 $\pm$ 0.62 kcal/mol | -50.52 $\pm$ 2.76 kcal/mol |

Table 1: Free Energy Changes