

Supplemental figures for **How Single Amino Acid Substitutions can Disrupt a Protein Hetero-Dimer Interface: Computational and Experimental Studies of the LigAB Dioxygenase from *Sphingobium* sp. Strain SYK-6**

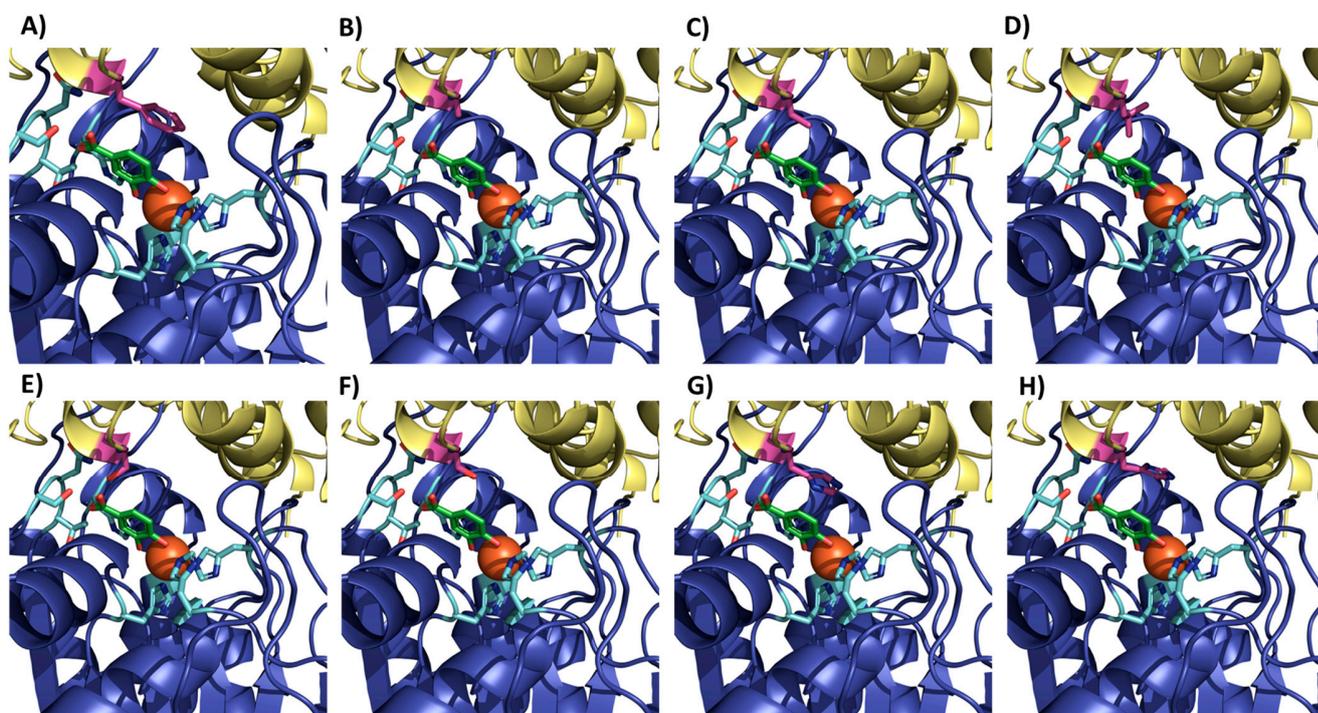


Figure S1: Active site of the LigAB complex with mutations at the 103 α residue. A close-up of the active site with the active site residues in yellow and F103 α residue in pink for (A) wild-type and corresponding mutations (B) alanine, (C) valine, (D) leucine, (E) serine, (F) threonine, and histidine protonated at the (G) δ or (H) ϵ nitrogen.

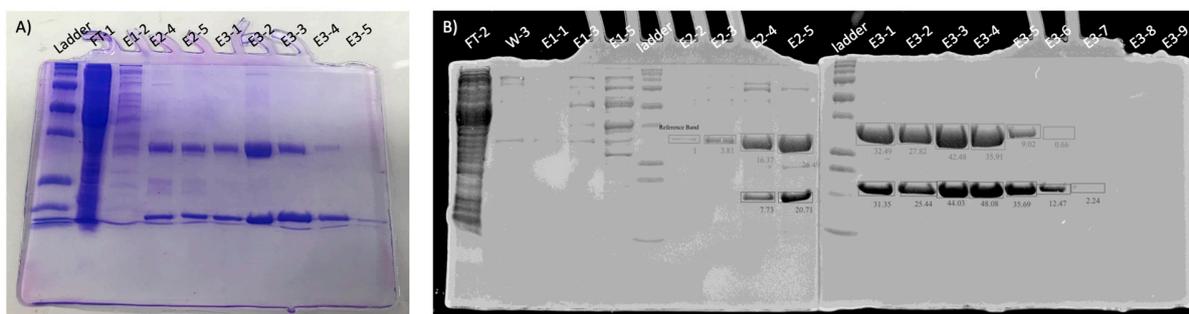
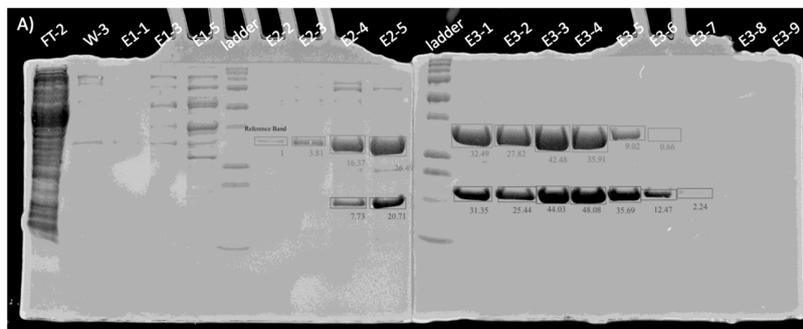


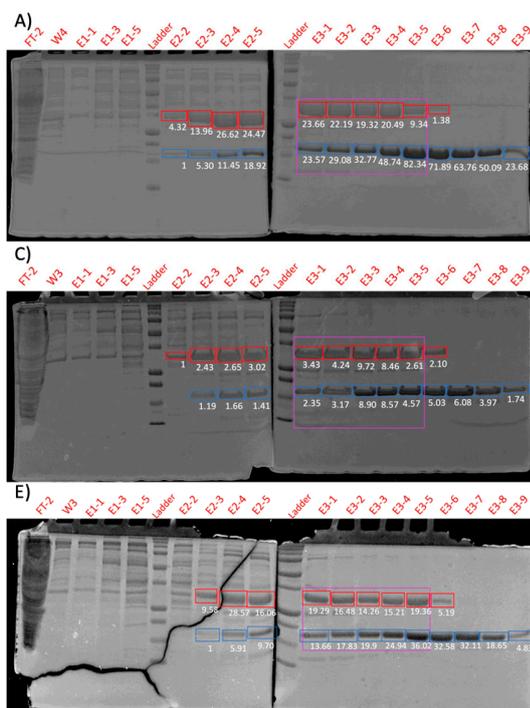
Figure S2: Wild-type LigAB purifications visualized by SDS-PAGE. A) A Representative SDS-PAGE gel of anaerobically purified LigAB and B) a SDS-PAGE gel of aerobically purified LigAB used in this study. Both gels were stained with Coomassie Blue.



B)

| wt | α | β |
|------|----------|---------|
| E3-1 | 49.1% | 50.9% |
| E3-2 | 47.8% | 52.2% |
| E3-3 | 50.9% | 49.1% |
| E3-4 | 57.2% | 42.8% |
| E3-5 | 79.8% | 20.2% |

Figure S3: Quantified percentages of α and β subunits from the wild-type LigAB purification. A) The SDS-PAGE gel from the purification of wild-type LigAB stained using Coomassie Blue. The reference band is marked and the weights of each of the bands are written on the gel. B) A table with the calculated percentages in fractions of LigA and LigB that are collected for concentration and used for protein characterization for wild-type LigAB.



B)

| F103A | α | β |
|-------|----------|---------|
| E3-1 | 49.0% | 50.1% |
| E3-2 | 56.7% | 43.3% |
| E3-3 | 62.9% | 37.1% |
| E3-4 | 70.4% | 29.6% |
| E3-5 | 89.8% | 10.2% |

D)

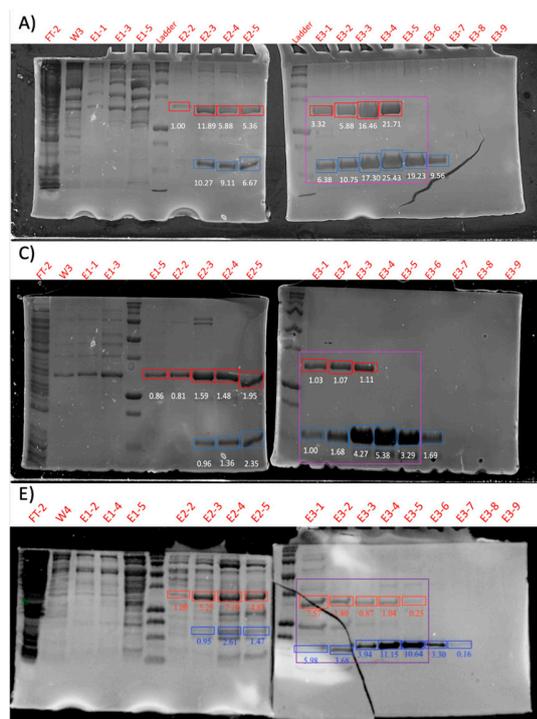
| F103V | α | β |
|-------|----------|---------|
| E3-1 | 40.7% | 59.3% |
| E3-2 | 42.8% | 57.2% |
| E3-3 | 47.8% | 52.2% |
| E3-4 | 50.3% | 49.7% |
| E3-5 | 63.6% | 36.4% |

F)

| F103L | α | β |
|-------|----------|---------|
| E3-1 | 41.5% | 58.5% |
| E3-2 | 52.0% | 48.0% |
| E3-3 | 58.3% | 41.3% |
| E3-4 | 62.1% | 37.9% |
| E3-5 | 65.0% | 35.0% |

Figure S4: Quantified percentages of α and β subunits from the nonpolar mutant purifications. : The SDS-PAGE gels stained with Coomassie Blue and tables with the percentages of LigA and LigB in fractions

that would be taken for further concentrating for further studies of nonpolar mutants: A) and B) F103A, C) and D) F103V, E) and F) F103L.



B)

| F103S | α | β |
|-------|----------|---------|
| E3-1 | 65.8% | 34.2% |
| E3-2 | 64.6% | 35.4% |
| E3-3 | 51.2% | 48.8% |
| E3-4 | 52.9% | 46.1% |
| E3-5 | 100.0% | 0.0% |

D)

| F103H | α | β |
|-------|----------|---------|
| E3-1 | 49.3% | 50.7% |
| E3-2 | 61.1% | 38.9% |
| E3-3 | 79.4% | 20.6% |
| E3-4 | 100.0% | 0.0% |
| E3-5 | 100.0% | 0.0% |

F)

| F103T | α | β |
|-------|----------|---------|
| E3-1 | 44.2% | 55.9% |
| E3-2 | 67.2% | 32.8% |
| E3-3 | 81.9% | 18.1% |
| E3-4 | 91.5% | 8.5% |
| E3-5 | 97.7% | 2.3% |

Figure S5: Quantified percentages of α and β subunits from the polar mutant purifications. : The SDS-PAGE gels stained with Coomassie Blue and tables with the percentages of LigA and LigB in fractions that would be taken for further concentrating for further studies of polar mutants: A) and B) F103S, C) and D) F103H, E) and F) F103T.

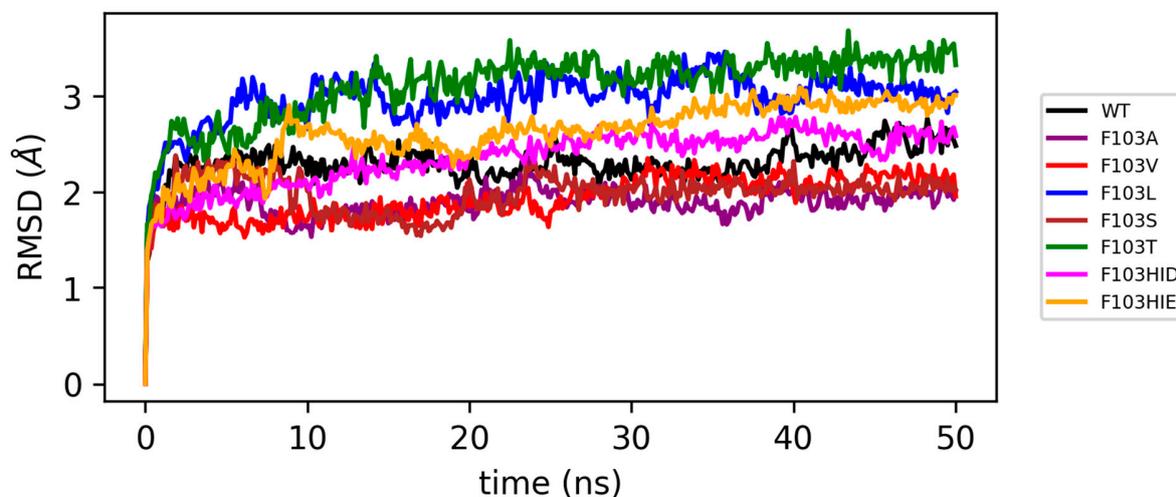


Figure S6: Backbone root mean square deviation of wild-type and F103 α mutants.. : RMSD of LigAB heterodimer relative to the equilibrated structure as a function of time. All variants equilibrate to under approximately 3.5 Å.

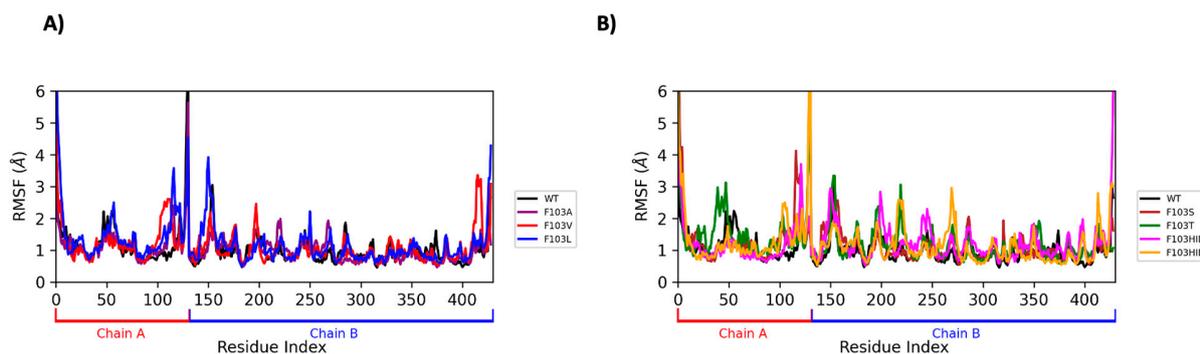


Figure S7: Root mean square fluctuations of wild-type and F103 α mutants.. : The average C_{α} root mean square fluctuation of the LigAB heterodimer. Residues in the A and B chain are labeled on the x axis. **(A)** Most differences in fluctuations occur adjacent to the mutation (residues >100) in chain A for variants alanine, valine, and leucine. **(B)** Whereas, fluctuations in chain B mainly occur in the polar residue variants including serine, threonine, and histidines.

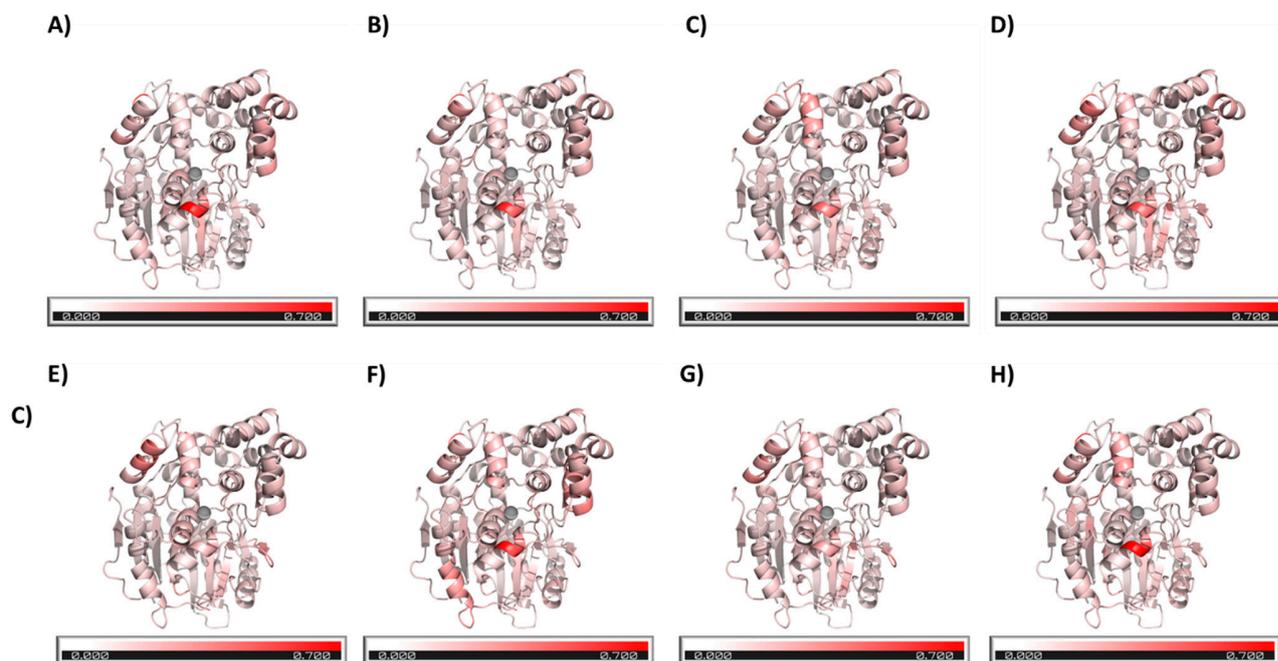


Figure S8: Calculated RMSF mapped to crystal structures of wild-type and F103 α mutants.. The average C α root mean square fluctuation of the LigAB heterodimer mapped onto the crystal structure for (A) wildtype, (B) alanine, (C) valine, (D) leucine, (E) serine, (F) threonine, and histidine protonated at the (G) δ or (H) ϵ nitrogen.

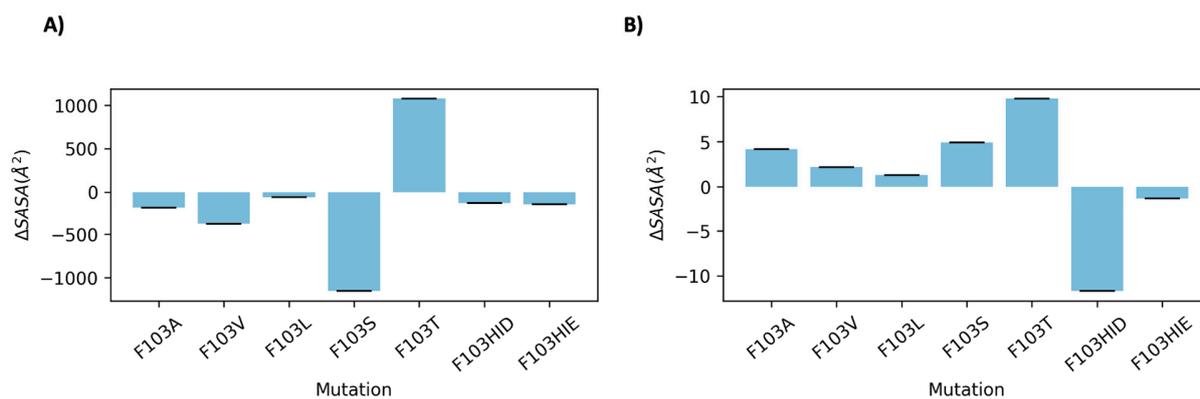


Figure S9: Solvent accessible surface area of F103 α mutants relative to wild-type (Δ SASA) (A) for the dimer as a whole and (B) for the difference for the individual mutated residues.