# Investigating the Stability of LigAB's Dimer Interface by Molecular Dynamics Simulations

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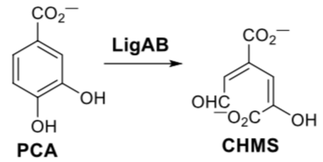
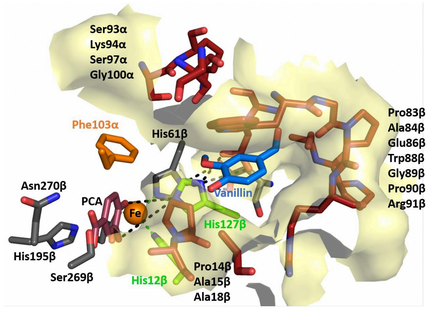
## Introduction

- Lignin, a carbon compound in cell walls, is underutilized
- LigAB catalyzes ring cleaving in lignin degradation pathway



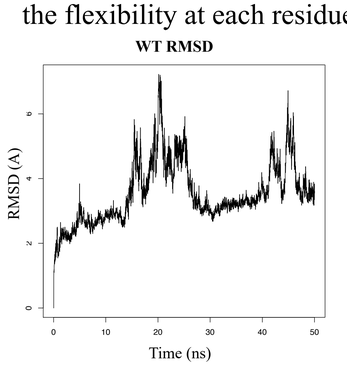

- LigAB is a homodimer of heterodimers
  - Both allosteric pocket and active site are proximal to dimer interface; dimer stability likely influences enzyme activity
  - Phe103α residue common to both of these sites

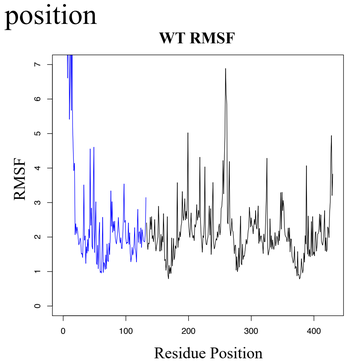



- Previously, some Phe103α mutants did not co-purify
  - Absence of a large, nonpolar residue disrupts interface
- Using GROMACS molecular dynamics package, stability of several Phe103α mutants computationally determined
- Calculated binding free energy of mutants with AMBER

## Wild Type

- Generated 50 ns trajectory for WT LigAB
  - Explicit solvent system and neutralized with $Na^+$
- Root Mean Squared Deviation (RMSD) shows deviation of the model from starting position over time
  - Relatively small values indicated accurate model
- Root Mean Squared Fluctuation (RMSF) used to determine the flexibility at each residue position



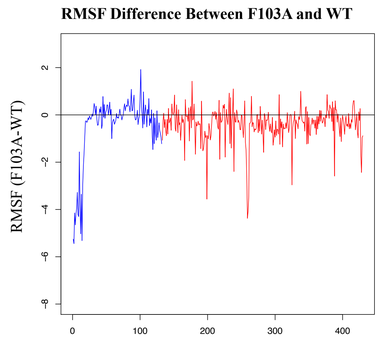



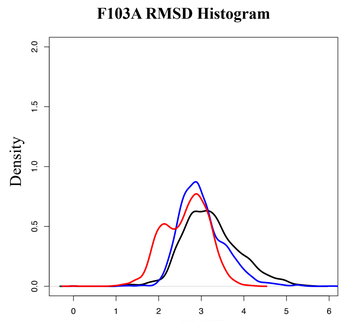

## Acknowledgements

I would like to thank Bakar Hassan for guiding me through what was a more arduous journey than I ever expected. I would also like to thank Jozafina Milicaj and Angelika Rafalowski for their help trying to find old data files that might not even exist. Lastly, of course I must thank Professor Taylor for her unending support both within and outside of the lab.

## F103A Mutant

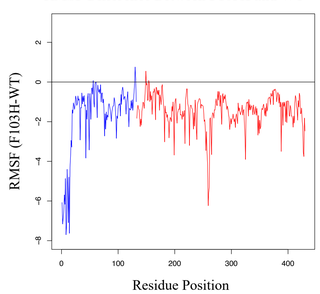
- F103A previously observed to not co-purify in expression screen
- RMSD histogram shows less deviation in beta subunit than alpha
  - Similar patterns to WT
  - Additional peak at lower RMSD for beta subunit
- RMSF of F103A smaller in beta subunit
  - Alpha mutation has global effects
- More stable than WT at residues in active site and allosteric pocket




- Mutation also caused changes in cross correlational interactions across the entire enzyme (data not shown)
- These data must be analyzed with experimental data to determine which interactions might be most important for maintaining dimer stability

## F103H Mutant




| Active Site Residue | RMSF Difference (Mutant - WT) | |
|---|---|---|
| | F103H | F103A |
| His12 | -1.24 | -0.34 |
| His61 | -1.79 | -0.81 |
| His127 | -4.17 | -2.57 |
| His195 | -1.32 | -0.55 |
| Leu197 | -1.06 | -0.22 |
| Glu242 | -0.44 | -0.17 |
| Ser269 | -0.95 | -0.03 |
| Asn270 | -1.00 | -0.23 |
| Thr271 | -1.68 | -0.47 |

- F103H showed similar patterns to F103A
  - Less flexible in beta subunit at comparable residue positions
  - Many peaks seem to correspond to active site residues or are within proximity of the site (data for both mutants displayed in table)

## References

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3. D.A. Case, R.M. Betz, D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke, A.W. Goetz, N. Homeyer, S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, C. Lin, T. Luchko, R. Luo, B. Madej, D. Mermelstein, K.M. Merz, G. Monard, H. Nguyen, H.T. Nguyen, I. Omelyan, A. Onufriev, D.R. Roe, A. Roitberg, C. Sagui, C.L. Simmerling, W.M. Botello-Smith, J. Swails, R.C. Walker, J. Wang, R.M. Wolf, X. Wu, L. Xiao and P.A. Kollman (2016), AMBER 2016, University of California, San Francisco.

## Free Energy Calculations

- Free energy calculations performed using MMPBSA function
  - Approximated dimer as receptor-ligand complex
- Binding free energy calculated by combining gas phase contributions with solvation free energy
- Used an implicit solvent system; Poisson Boltzmann (PB)
  - Represent solvent as continuous medium
  - Describes electrostatic environment in solvent with ions

$$\vec{\nabla}\cdot\left[\epsilon(\vec{r})\vec{\nabla}\Psi(\vec{r})\right] = -4\pi\rho^f(\vec{r}) - 4\pi\sum_i c_i^\infty z_i q\lambda(\vec{r})e^{\frac{-z_i q\Psi(\vec{r})}{kT}}$$

| | WT | | F103A | |
|---|---|---|---|---|
| Energy Component | 10-30ns | 30-50ns | 10-30ns | 30-50ns |
| Van der Waals | -211.01 | -221.05 | -195.38 | -195.67 |
| Electrostatic | -556.66 | -608.08 | -407.44 | -473.36 |
| Electrostatic - Solvation (PB) | 634.34 | 687.19 | 493.87 | 564.51 |
| Non Polar Cavity Formation | -147.59 | -155.41 | -134.42 | -137.91 |
| Non Polar Dispersion | 286.39 | 296.42 | 262.05 | 267.45 |
| Total Binding Free Energy | 5.465 | -0.936 | 18.676 | 26.023 |

- Total binding free energy of F103A is significantly less stable than WT; not within range of standard deviations
  - Confirms original hypothesis
- Electrostatic interactions overall weaker in F103A
  - Both attractive and repulsive forces
  - Might implicate water in stability of the dimer interface or disruption of the stabilizing interactions

## Future Directions

- Trajectories of at least 50 ns will be generated for all previously studied Phe103α mutants
- Experimental data will be collected for each mutant
  - Aerobic purification and corresponding SDS-PAGE gels
    - Example below shows F103S; beta subunit (32 kDa) elutes without the alpha subunit (18 kDa)
  - Resulting gels will be quantified to determine co-purification of respective mutants
- Gives insight on which interactions prevent dimer formation