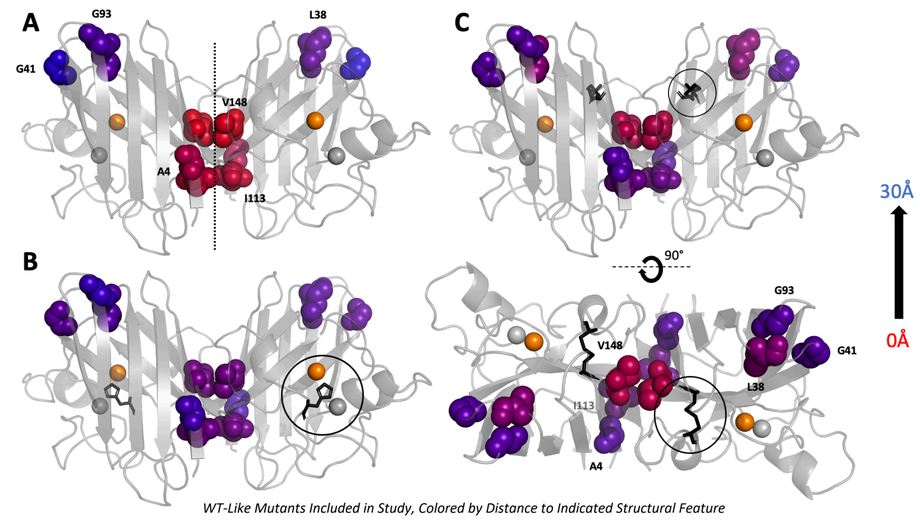
# ALS-Causing SOD1 Mutations Cause Common Perturbations to Maturational Free Energy

Nick Wells, Colin Smith
Department of Chemistry, Wesleyan University, Middletown CT 06457

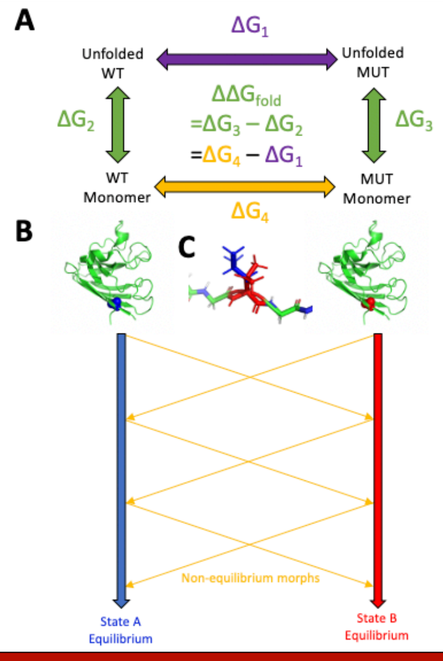
## Introduction




*WT-Like Mutants Included in Study, Colored by Distance to Indicated Structural Feature*

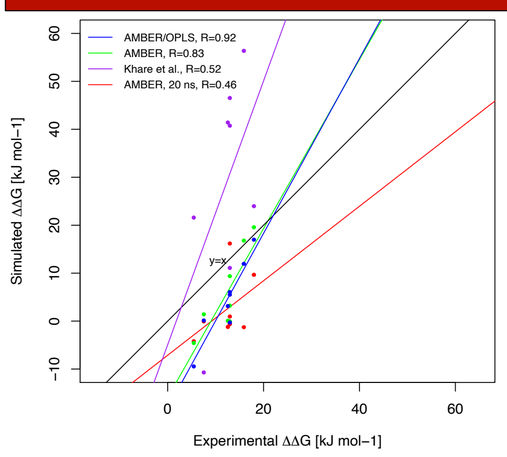
- Approximately 20% of familial ALS cases are linked to mutations in the SOD1 gene[1]
- Immature SOD1 is implicated in misfolding, aggregation, and disease progression
- Distinct and conservative "wild type-like" (WT-like) variants exhibit no obvious patterns of structural or functional change, despite causing similar phenotypic disorder (ALS)
- MD Free energy calculations offer insight into how WT-like variants similarly influence SOD1 maturation to cause disease

## Methods



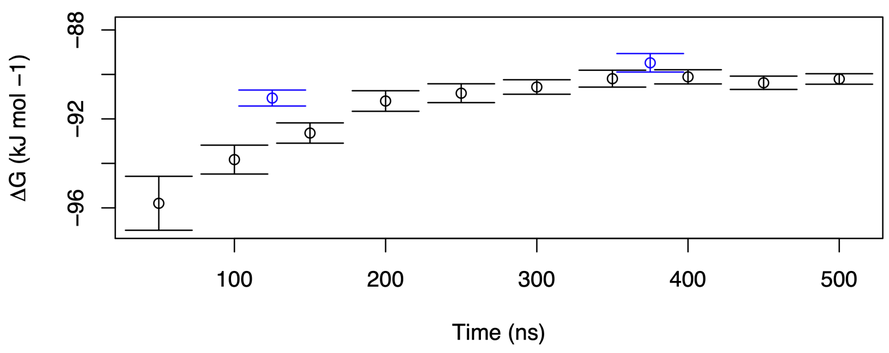

- Robust alchemical mutation workflow was adopted to calculate ΔG of a mutation on a state change ($\Delta G^{mut}_{transition}$)[4]
- 3x 500 ns trajectories generated for each WT-like variant studied
- Snapshots extracted every 1 ns from trajectories, and hybrid residues with WT & MUT sidechain atoms are built into the *pdb* structure (**C**)
- The work is integrated by deriving the δH/δλ curve generated by each morph simulation for the forward (λ=0->1) and reverse (λ=1->0) to find $\Delta G_{mut}$ values
- $\Delta G_{mut}$ values at different states are compared to find $\Delta\Delta G^{mut}_{transition}$

## Methodological Validation




- AMBER $\Delta\Delta G_{dim}$ values (green) correlate to experimental values better than previous studies (purple) (R = 0.83 vs 0.52)
- AMBER/OPLSAA value averaging (blue) further improves correlation (R = 0.92)
- Using only 20ns of AMBER trajectories (red) worsens correlation (R = 0.46), demonstrating need for increased sampling over previous studies

## ns-μs Timescale Motion in SOD1

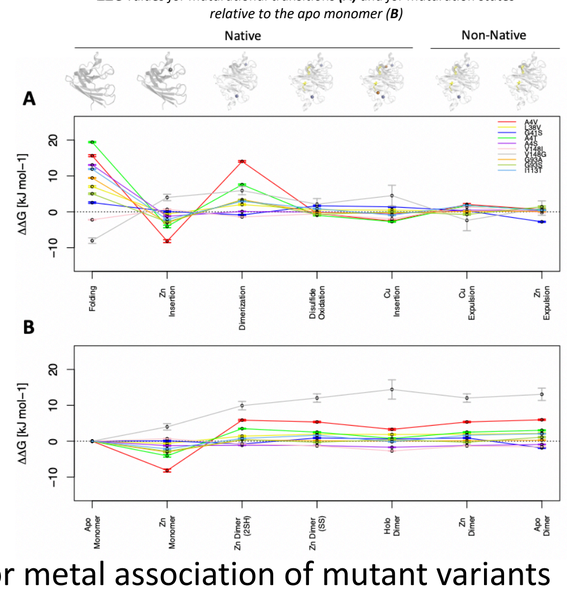



- Cumulative (black) $\Delta G_{A4V}$ values in apo dimer converge to be more in agreement with experiment after ~300ns
- Discrete values (blue) show a significant change in $\Delta G_{A4V,}$ demonstrating the need for long trajectories to sample ns-μs timescale motion for accurate free energy calculations

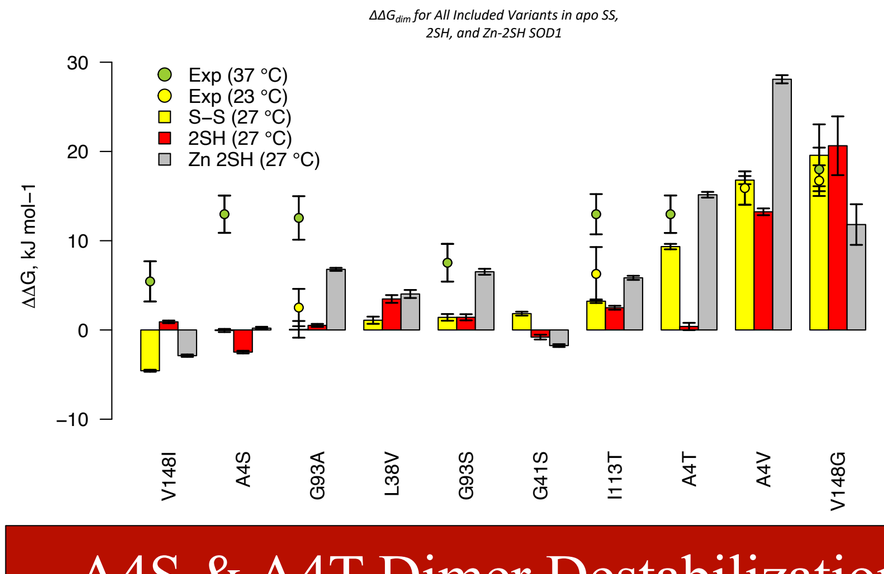
## Trends in Maturational ΔG Perturbation

- Most WT-like variants significantly destabilize folding and dimerization, contributing to the prevalence of immature, disease-prone SOD1 conformers
- Free energy perturbations are larger in magnitude for earlier native maturational transitions
- Non-native maturation influenced by WT-like variants, though pattern unclear
- WT-like variants tend to thermodynamically stabilize metalation, perhaps indicating $Cu^{2+}$ chaperone (Ccs1) interaction disruption to explain poor metal association of mutant variants

*ΔΔG values for maturational transitions (**A**) and for maturation states relative to the apo monomer (**B**)*



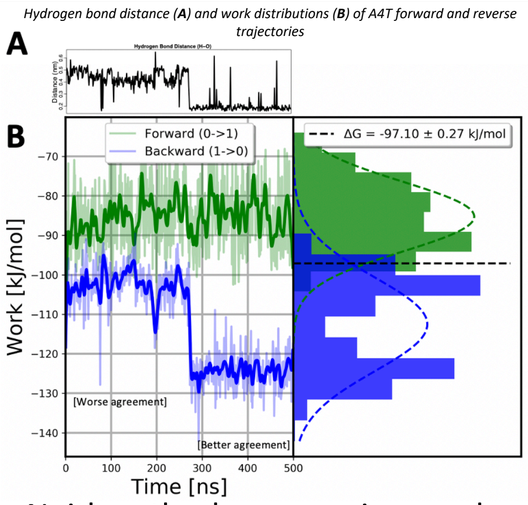

## Common Perturbations to Dimerization




- $\Delta\Delta G_{dim}$ values for most WT-like Variants depends on disulfide oxidation and metalation state
- $Zn^{2+}$ binding increases magnitude of perturbation over apo-$SOD1^{2SH}$ in most cases

## A4S & A4T Dimer Destabilization Mechanism

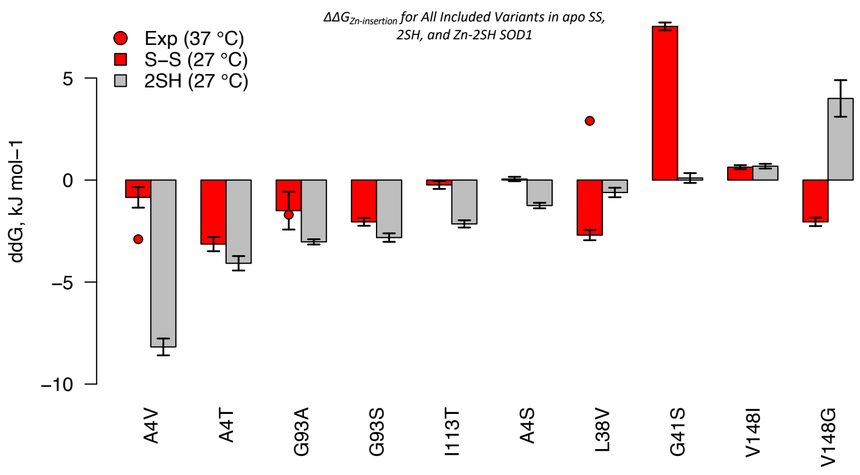
*Hydrogen bond distance (**A**) and work distributions (**B**) of A4T forward and reverse trajectories*




- $\Delta\Delta G_{dim}$ for A4T is closer to the experimental value than A4S
- A4T work distributions show one mode which does not correspond to experimental and one which does (**B**)
- A4S shows less sampling of the first mode
- Hydrogen bonding between Thr4 and Gly150 in MUT monomer trajectory present where work values align better with experiment (**A**)
- Neither the less agreeing mode nor hydrogen bonding are present in dimer trajectories of A4S or A4T
- We propose this hydrogen bond stabilizes the monomer but not dimer, increasing $\Delta\Delta G_{dim}$

## Common Perturbations to $Zn^{2+}$ Binding

- $Zn^{2+}$ insertion is stabilized by most WT-like variants, and $Zn^{2+}$ partially rescues from folding destabilization of most mutants
- Disulfide reduction increases the stabilization of $Zn^{2+}$ insertion in most cases




- Perturbations to metal insertion are largely long-distance (15-30 Å), indicating the importance of allostery in SOD1 metal binding

## Conclusions and Future Directions

- Perturbations to the maturational free energy landscape of SOD1 offer a unifying effect of all ALS-causing, WT-like variants
- Large-scale MD simulations are needed to sufficiently probe the conformational space of SOD1 for accurate free energy calculations
- Folding and dimerization are mostly destabilized by mutants, while metalation is mostly stabilized
- Non-native maturation is not especially affected by WT-like mutations
- Dimerization-destabilizing effects of mutants are more pronounced in transitions which correspond to SOD1's biological maturation pathway
- A4S and A4T residues exhibit putative hydrogen bonding to the dimerization site-adjacent backbone of SOD1, providing a possible mechanism of dimer destabilization
- $Zn^{2+}$ insertion partially rescues SOD1 from the folding destabilization of most WT-like mutations, potentially guiding future therapeutic efforts
- Identification and characterization of an innate allosteric pathway which allows $Zn^{2+}$ binding to stabilize the protein is needed
- Identification and characterization of similar allosteric pathways used by distinct WT-like mutations by RMSF, dihedral, correlated motion analysis is needed

## References and Acknowledgements

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**References**

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