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Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto

Changing the Paradigm in Petroleum Industry: Enhancing the catalytic rate of DszD by QM/MM calculations

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1. Background

1. Background

The problem:

- Sulfur compounds are present in crude oil in concentrations between 0.1 and 8% (w/w).
- Legal restrictions in increasingly more nations regarding the sulfur content in fossil fuels.
- The main method to desulfurize crude oil is the energetically expensive chemical hydrodesulfurization (HDS).
  - High temperature and high pressure

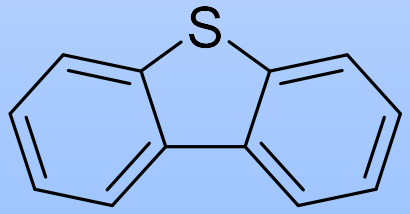


Fig 1. Chemical structure of dibenzothiophene (DBT)

1. Background

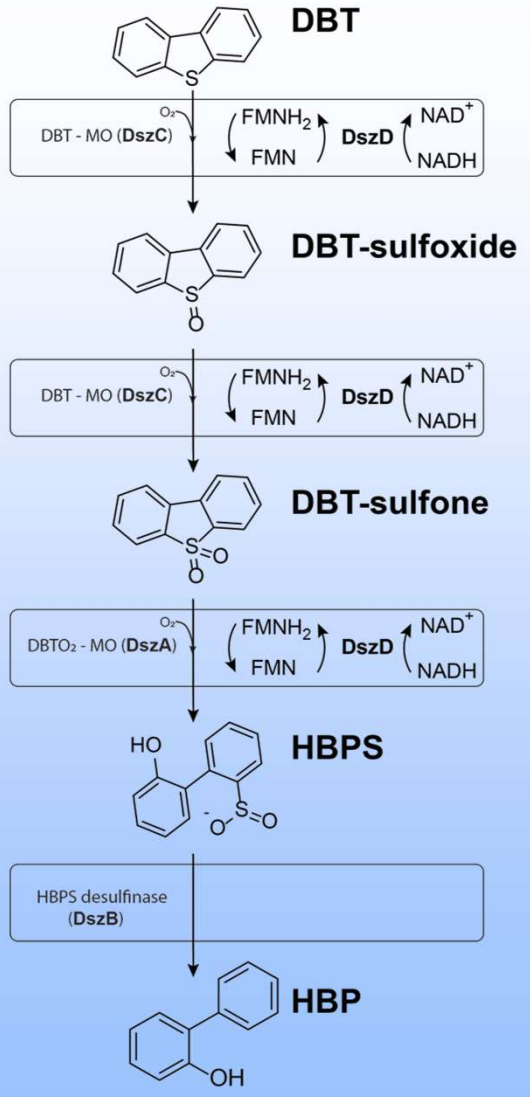
The alternative:

- Biocatalytic desulfurization (BDS)
  - Explores the process "4S pathway" of *Rhodococcus erythropolis*
    - Uses DBT as a source of sulfur
  - Maintains the energetic value of the oil
  - Much cheaper than HDS
  - Does not produce undesirable by products
  - **PROBLEM:**
    - Catalytic rate not attractive for industrial application

1. Background

4S pathway

- Desulfurization of DBT to 2'-hydroxybiphenyl
- Carried out by four enzymes of *Rhodococcus erythropolis*:
  - DszA
  - DszB
  - DszC
  - DszD



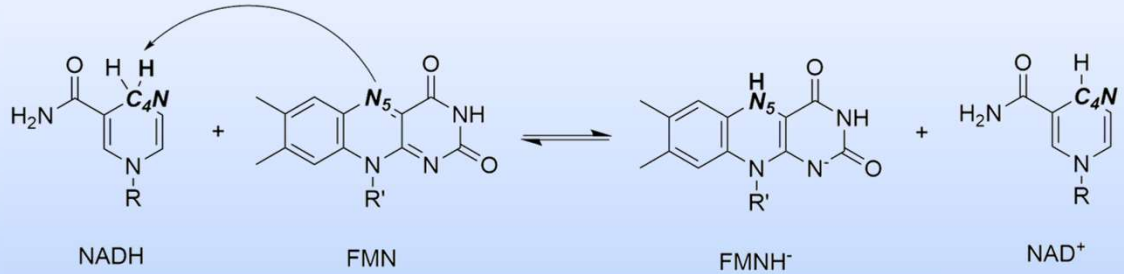
1. Background

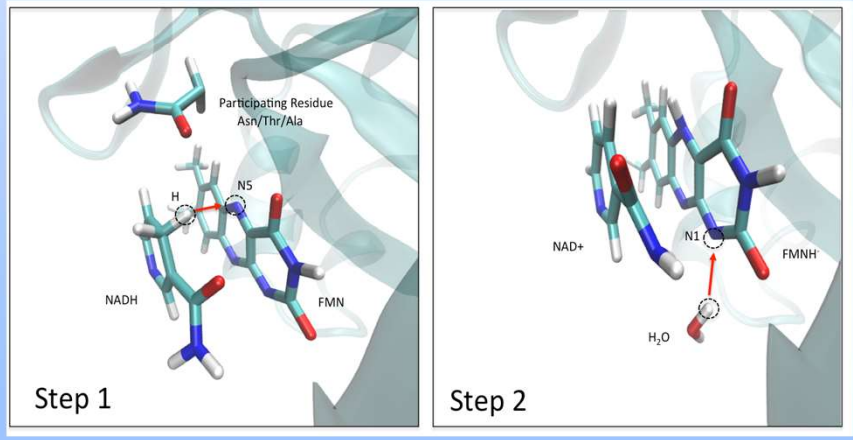
DszD

- Responsible for supplying $FMNH_2$ in the 4S pathway
- Overexpression of DszD improves the catalytic rate of the whole pathway
- Experimental studies revealed the importance of Thr^{62}
- Mutation of Thr62 by Asn and Thr improved the catalytic rate

1. Background

DszD

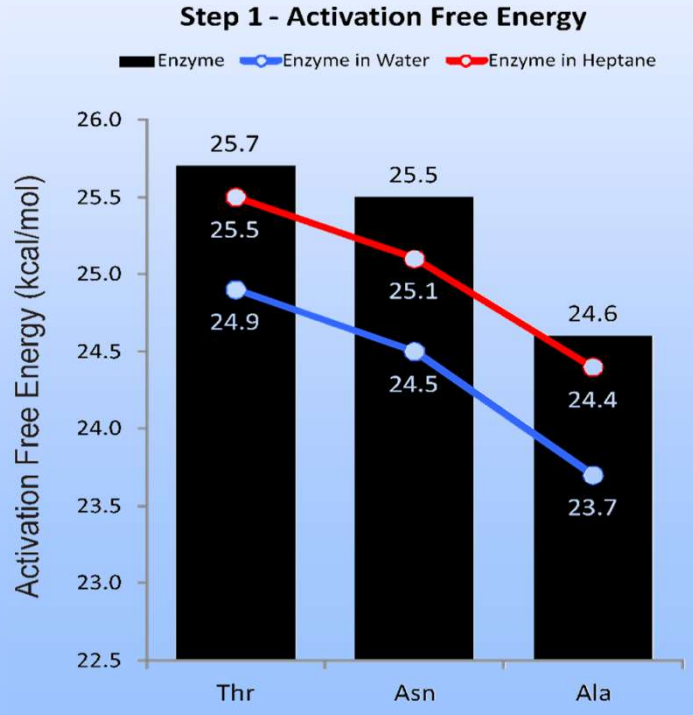




Sérgio F. Sousa et al. *The Journal of Physical Chemistry A* **2016** *120* (27), 5300-5306

1. Background

DszD



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1. Background

Goal

- Attempt to find ways to accelerate the limiting step of the DszD reaction using hybrid quantum mechanics/molecular mechanics (QM/MM) methods, by systematic mutation of Thr62 for 18 different amino acid residues.

2. Methods

2. Methods

Mutations

- Mutation of the participating residue by all amino acids
- Using pymol mutation feature
- *Dunbrack rotamer library* of rotamers

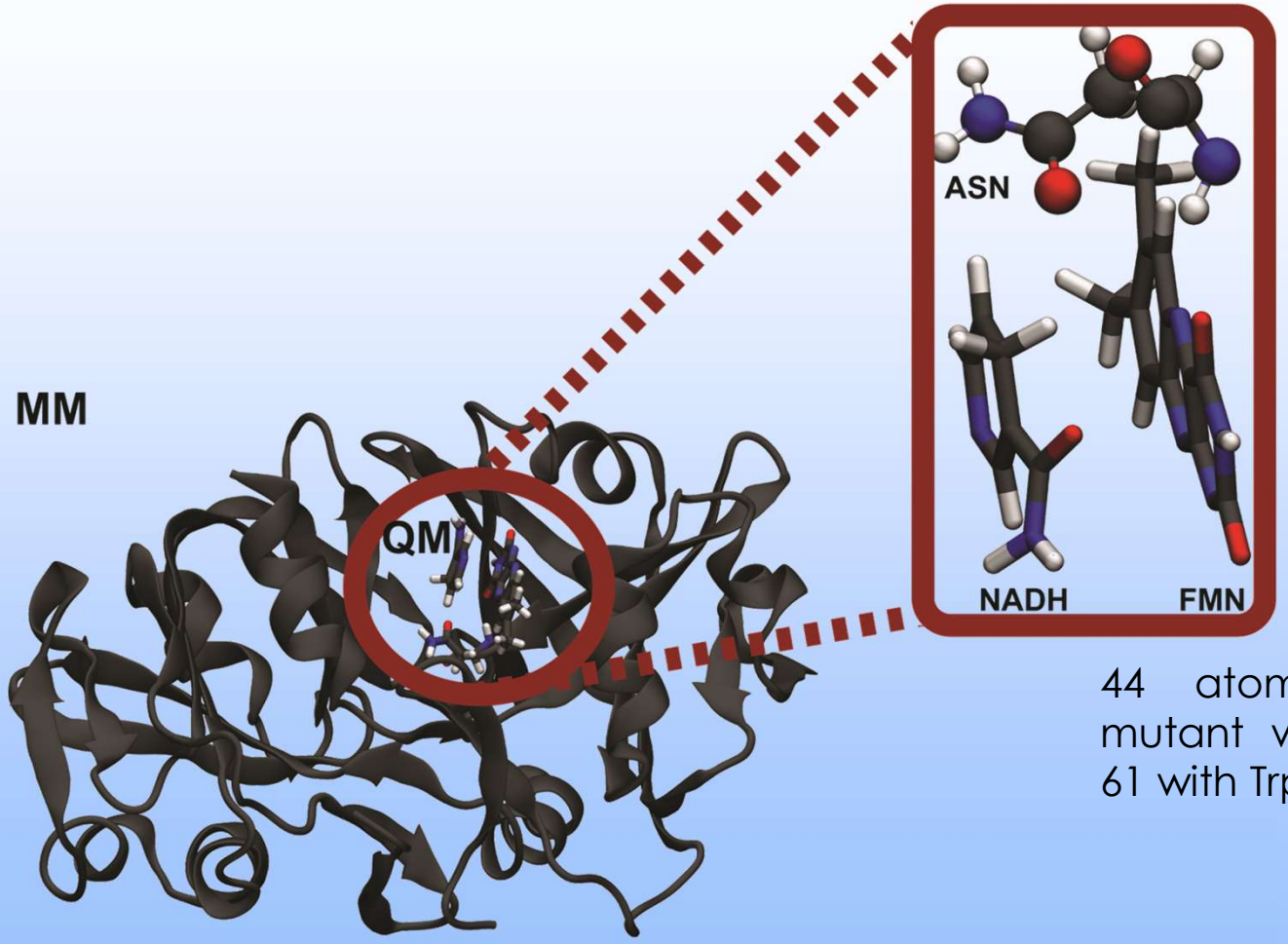
Minimization

- Minimization of the mutated residue

ONIOM

- Optimization of the reactants
- Linear transit scan of the rate limiting step
- Characterization of the transition state
- Intrinsic reaction coordinate (IRC) calculations to find the mínima
- Final energies at B3LYP-D3/6–311++G(2d,2p):FF99SB//B3LYP/6–31G(d):FF99SB

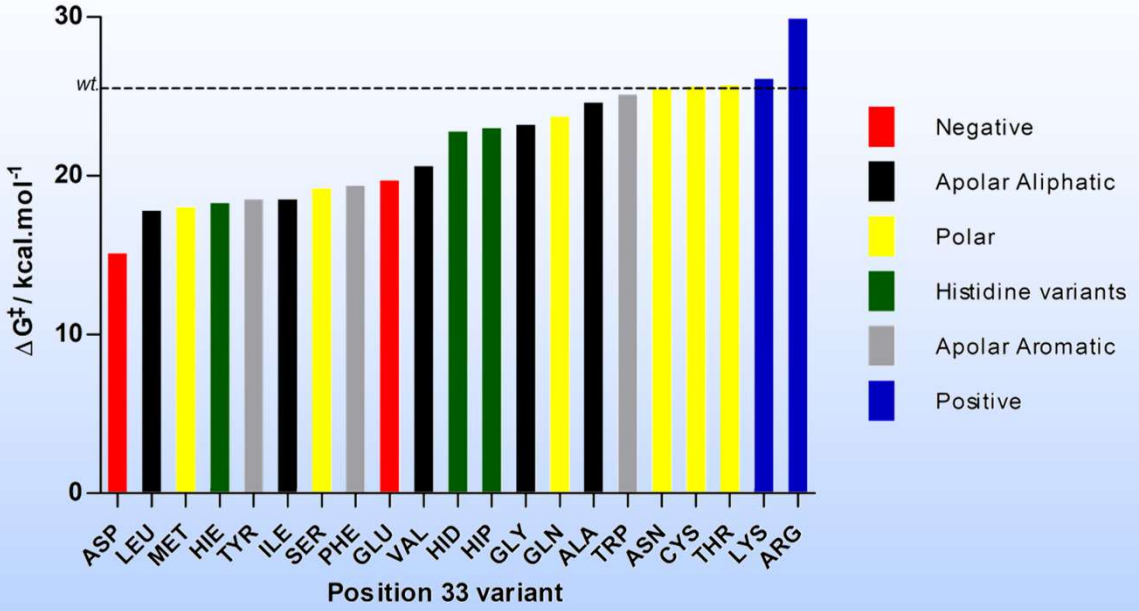
2. Methods



44 atoms in the mutant with Gly to 61 with Trp

3. Results

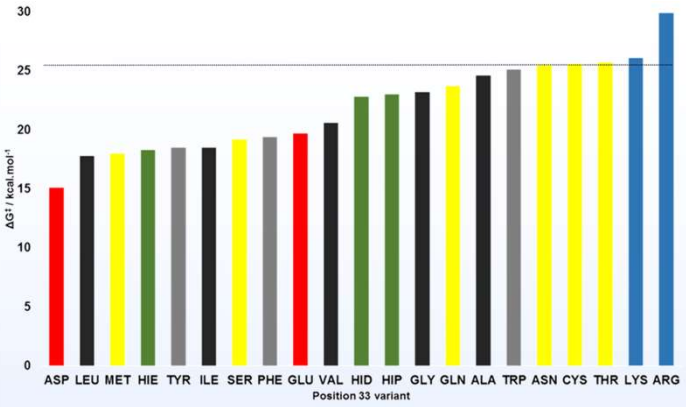
3. Results

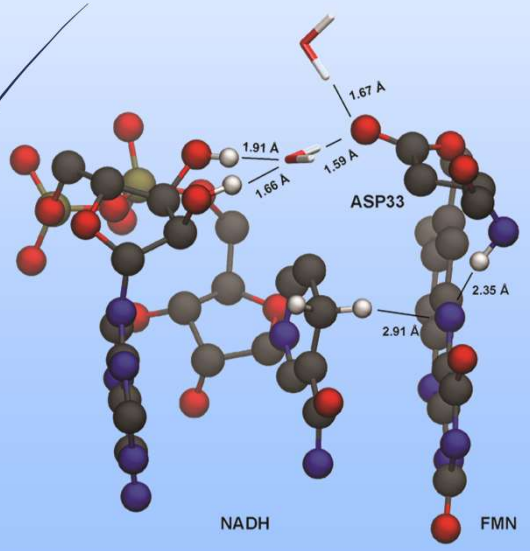


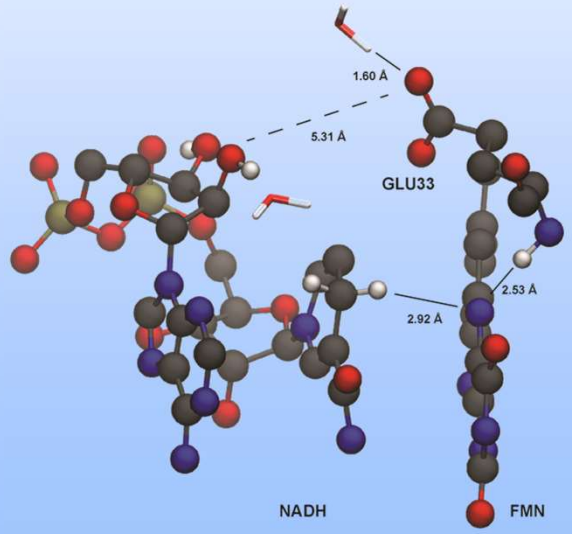
- The results confirm the potential that the reaction rate of DszD has to be enhanced through
- A direct correlation between the "type" of the residue and the activation free energy of the reaction cannot be drawn

3. Results

Structural analysis



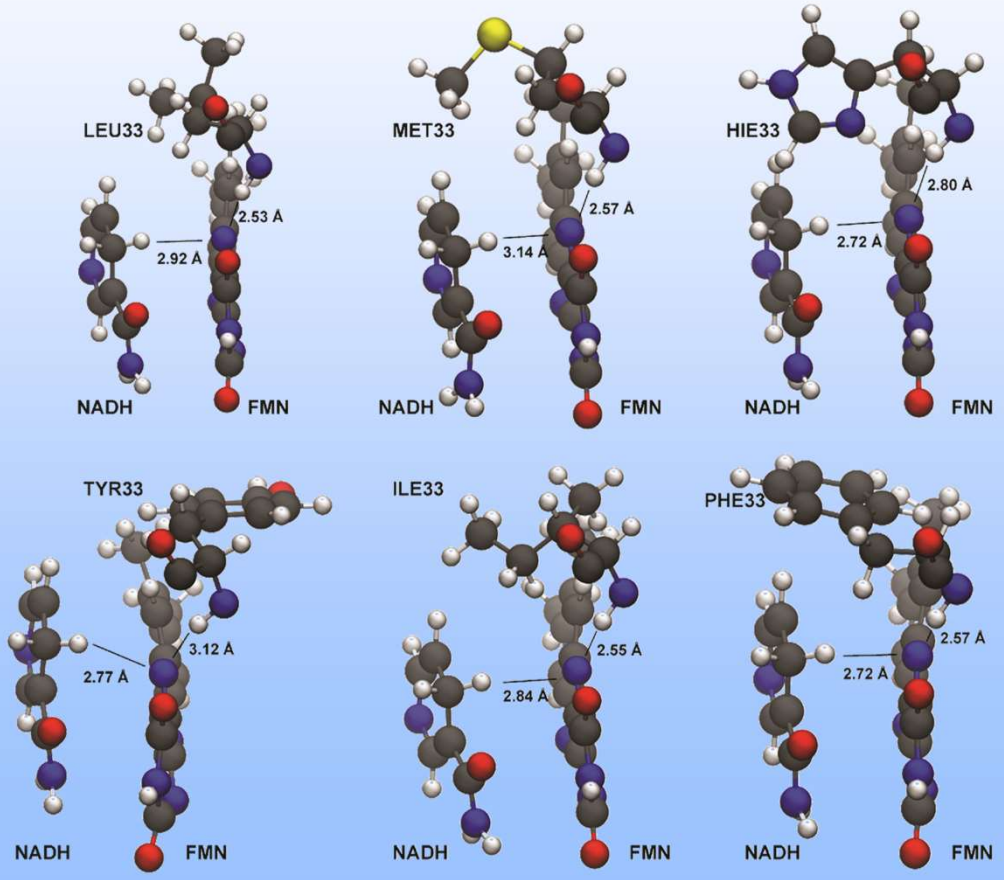


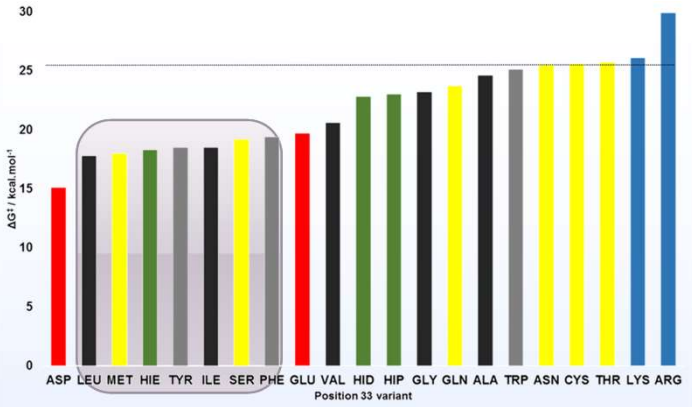


- Negative charge may stabilize NAD^+
- Hydrogen bonding system between water, ASP and NAD^+ is unique
- GLU is farther from NADH than ASP which may explain the higher activation barrier

3. Results

Structural analysis

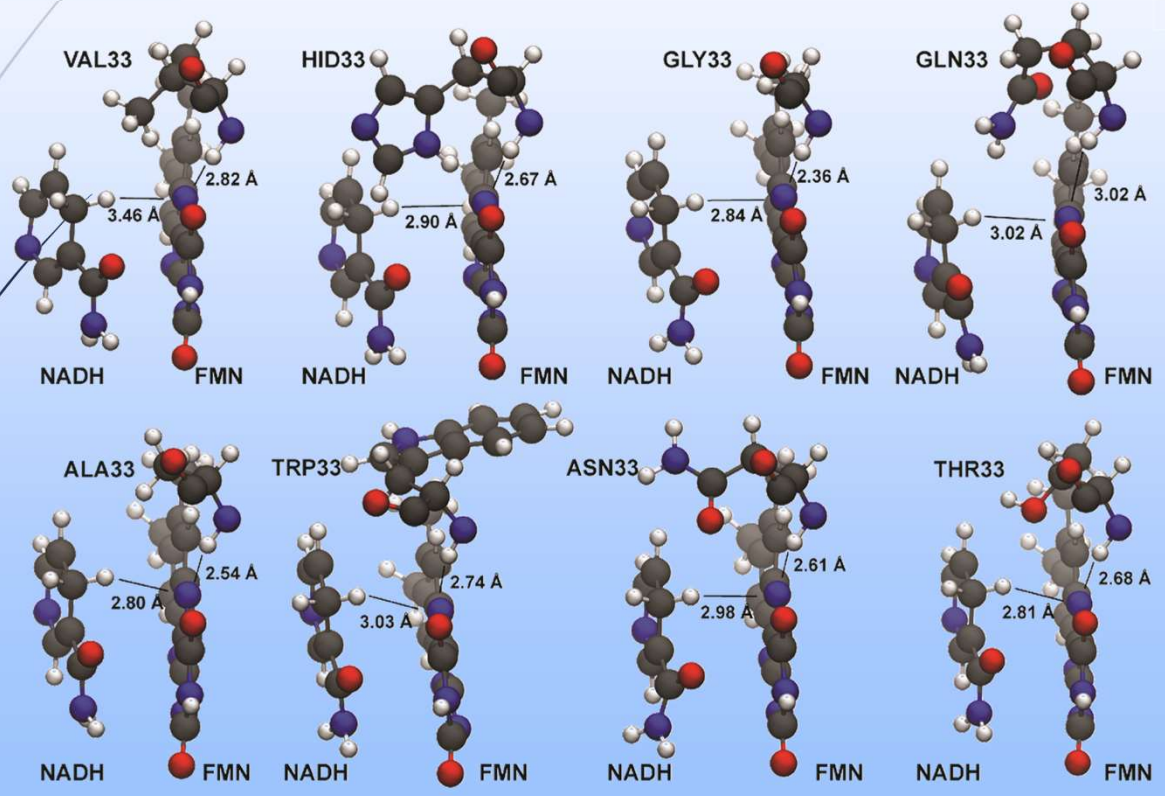


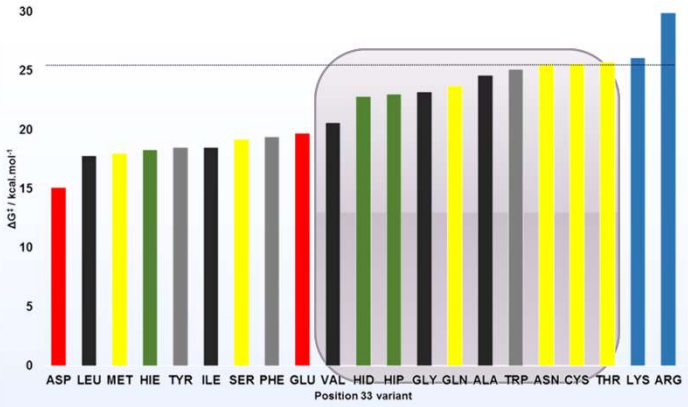


- Side chains stay far from the reaction coordinate
- Conformation of the side chains seem to lock NADH in place.
- Hydrogen bond NH—N5 in the variant with TYR is the weakest of all variants → less stabilization of the reactants structure → reactants and TS energetically closer

3. Results

Structural analysis

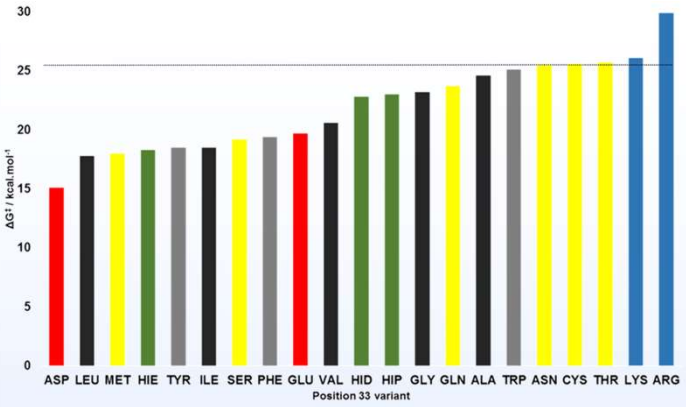


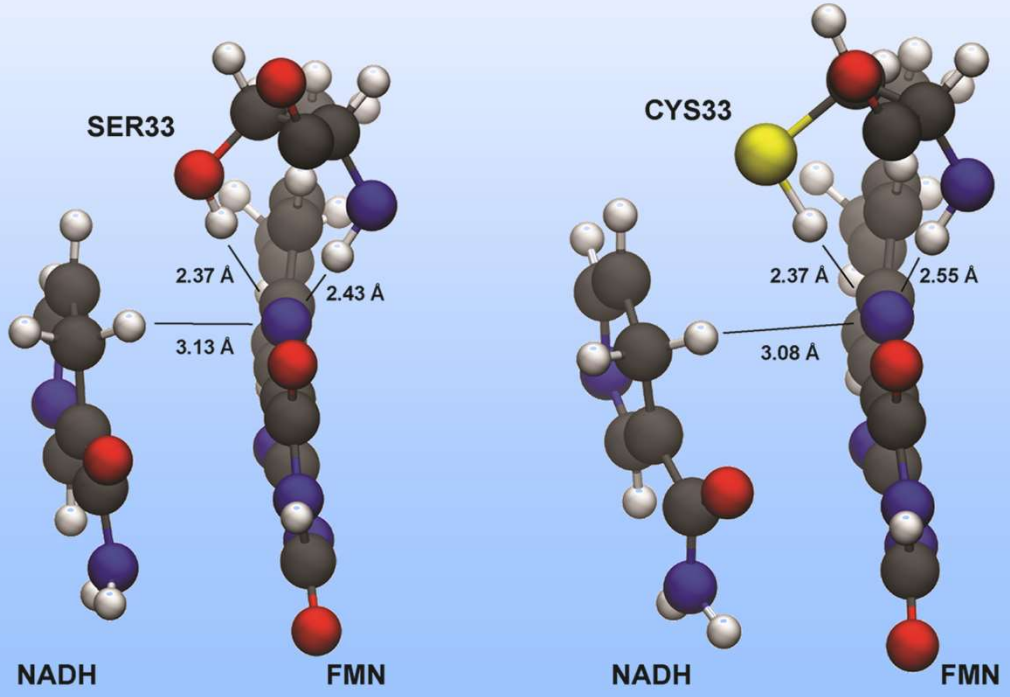


- Short or no side chains like those of VAL, ALA and GLY seem to let NADH misalign with respect to FMN
- Polar groups directly pointing to the reaction coordinate, may impair the free flow of the electron to be transferred

3. Results

Structural analysis

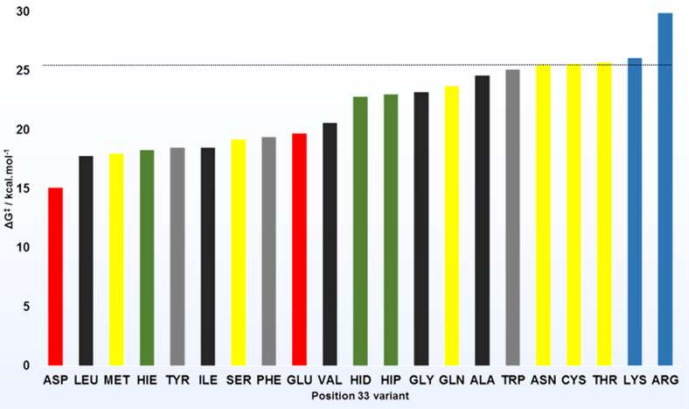


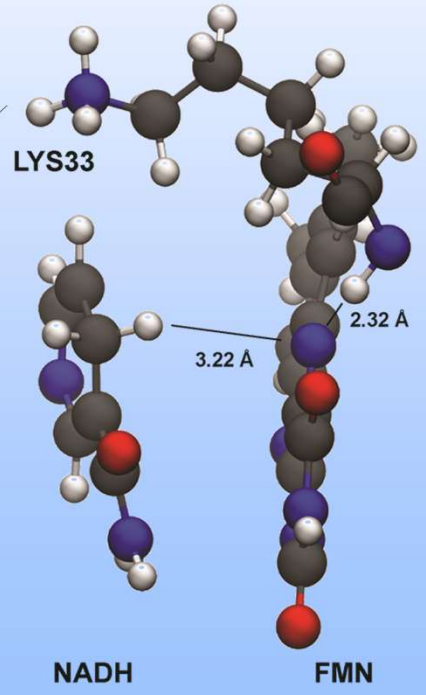


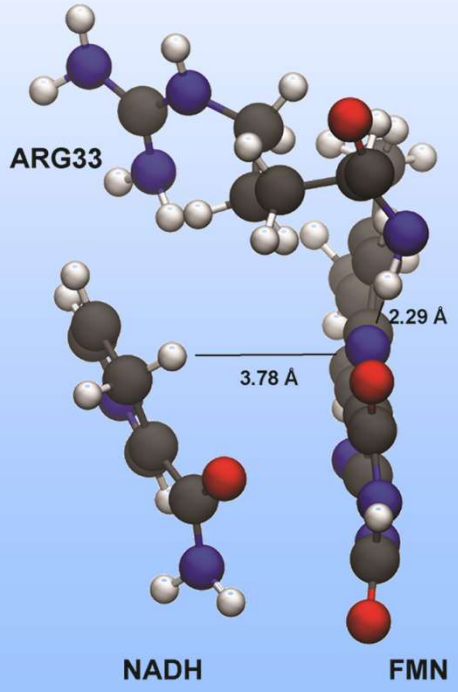
- Misalignment of NADH towards FMN in the variant with CYS
- Hydrogen bond between the NH_{res33} – $N5_{FMN}$ weakens more from R → TS with CYS (2.55 Å –> 2.74 Å) than with SER (2.43 Å –> 2.54 Å)
- Hydrogen bond OH_{Ser} – $N5_{FMN}$ is stronger in the TS than in the reactants

3. Results

Structural analysis



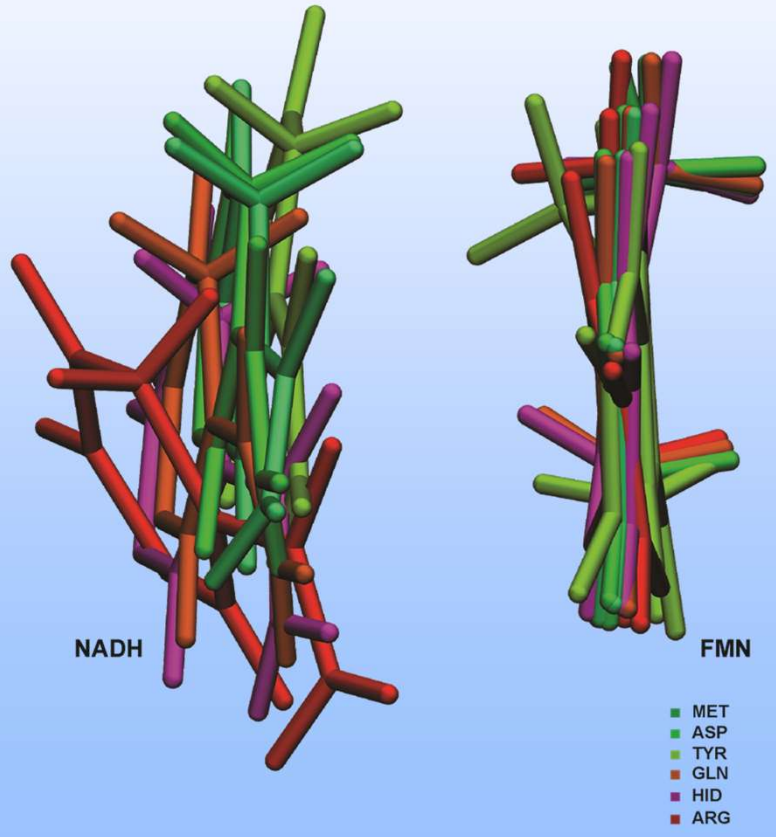


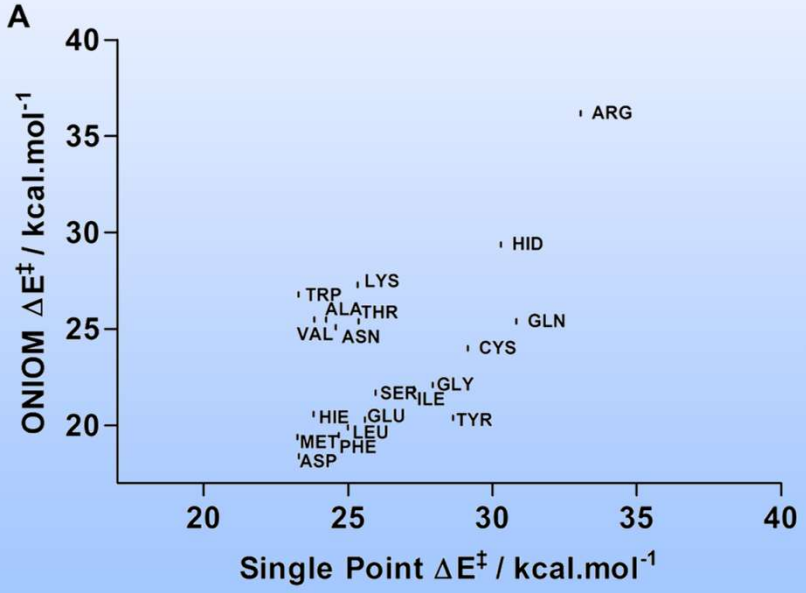


- Positive charge of these residues are pointing to NADH which is oxidized to NAD+ with the loss of the hydride
- Very bulky residues

3. Results

Structural analysis





4. Conclusions

4. Conclusions

- The spectator residue seems to slow down the reaction
- The catalytic rate of DszD can be greatly enhanced through point mutations of the spectator residue
- Enhancement of the other enzymes of the 4S pathway is mandatory to make the 4S pathway industrially attractive comparatively to the chemical processes currently used.

5. Acknowledgements

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