Ectoine synthase (EctC) - Fe(II) binding site parametrization

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Outline

1. Brief introduction to the enzyme and catalyzed reaction
2. Motivation for parameterization
3. DFT optimization
4. ADMP
5. Methods description
6. Parameters & their evaluation
7. Conclusions

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Ectoine synthase and Ectoine

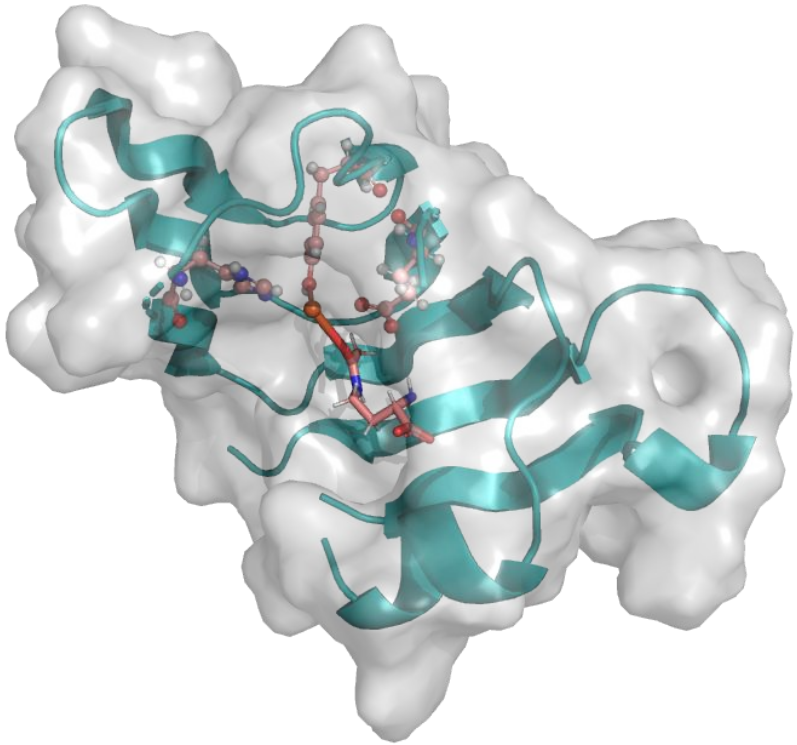


Fig. 1 *P. laucus* EctC crystal structure with catalytic core exposed. Fe(II) ion with ligands (Glu57, Tyr84, His92 and N-γ-acetyl-2,4-diaminobutyrate) are visible inside the pocket. PDB: 5ONN[1,2]

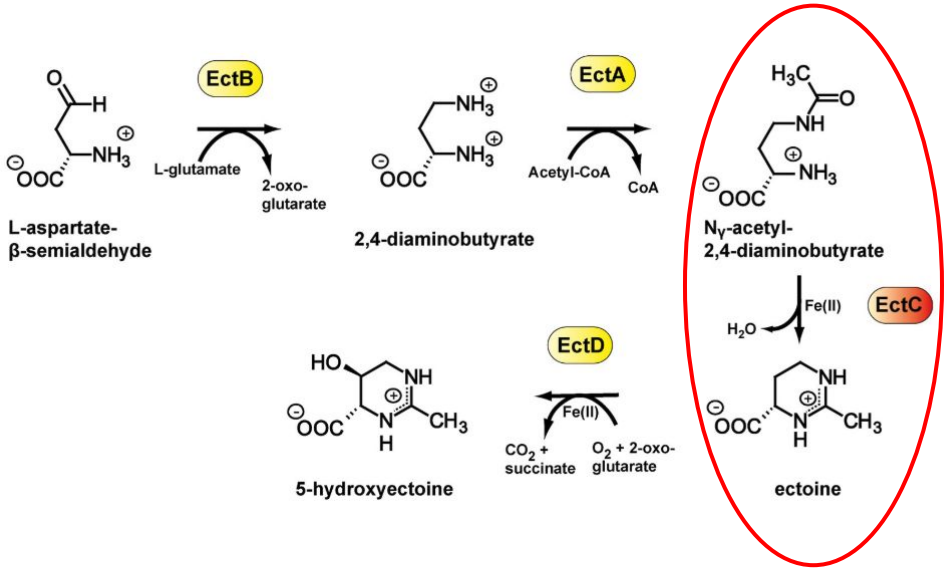
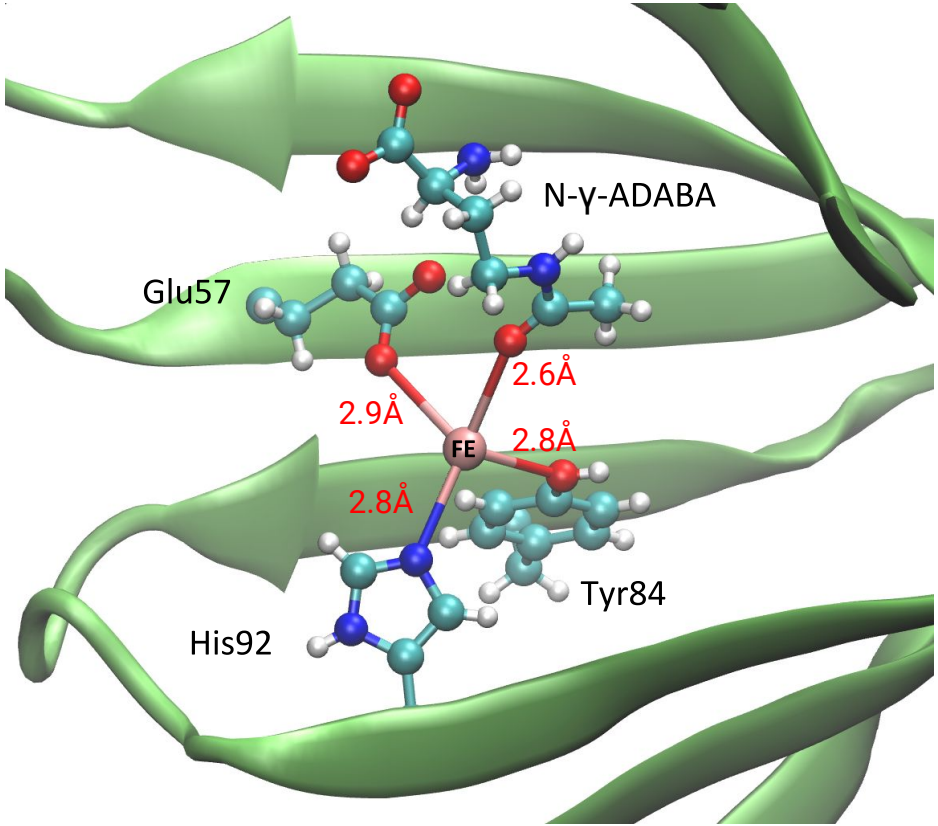


Fig. 2 Pathway of the ectoine biosynthesis. Cyclocondensation reaction of N-γ-ADABA marked with red circle[3].

Motivation for the parametrization



- Fe - L bonds too long
- geometry refinement needed before QM and QM/MM calculations
- reliable parameters necessary for MD simulation

Fig. 3 Model of Fe(II) binding site cut from 5ONN[1] crystal structure. Fe - L distances shown in red.

DFT/B3LYP optimization

E_5+ 36.7 kcal/mol

E_5+ 18.3 kcal/mol

E_5- reference

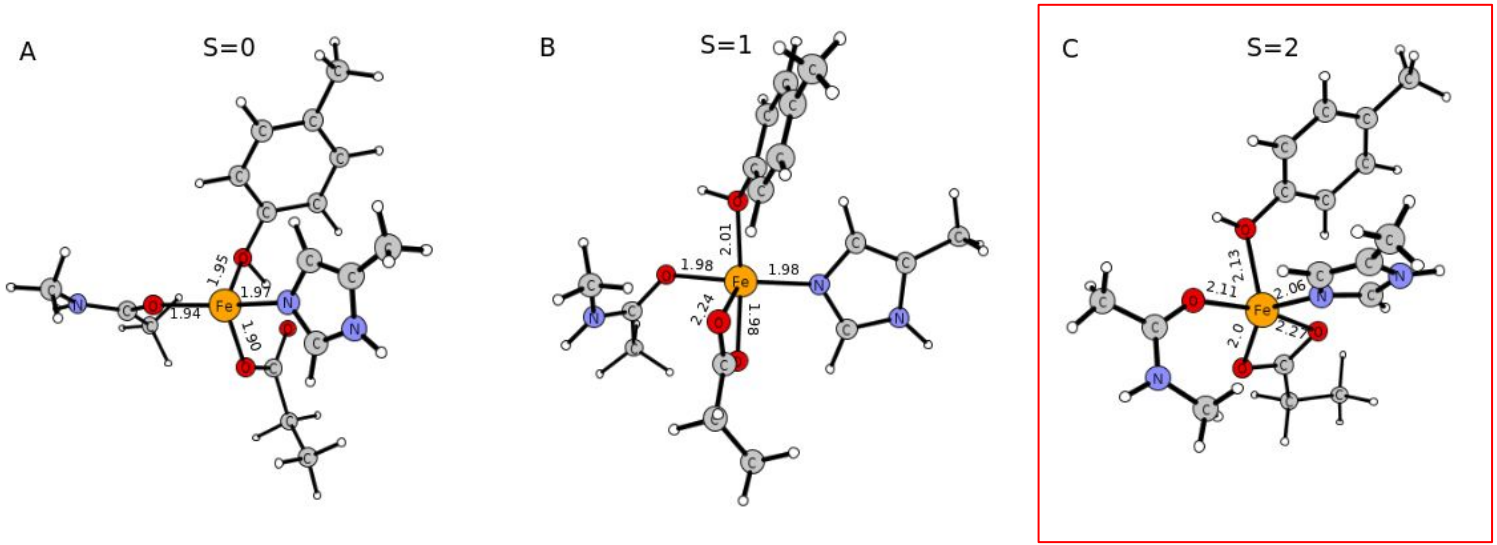


Fig. 4 Three optimized geometries with Fe(II) in different spin-state: A) singlet, B) triplet i C) quintet. Calculated with DFT: B3LYP-D3/Def2TZVP.

ADMP - quintet model

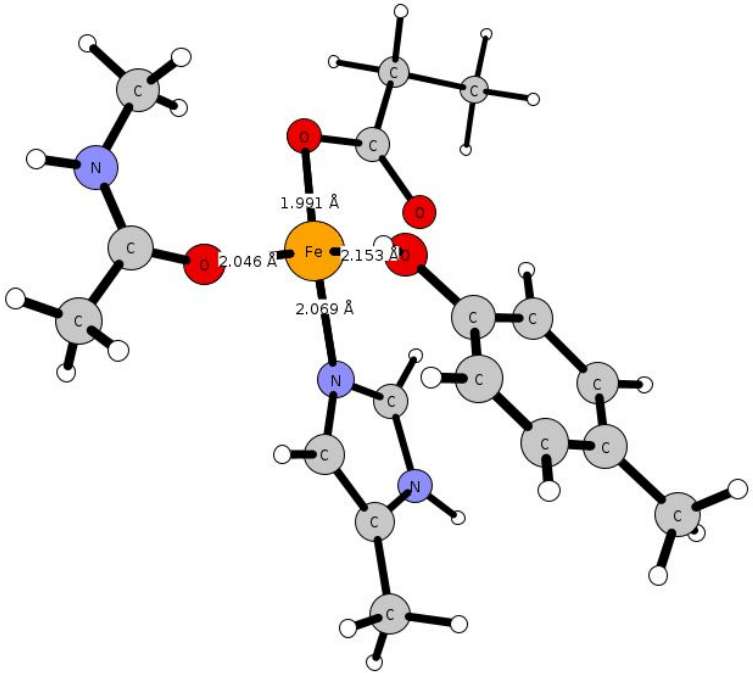


Fig. 5 Reference trajectory (149 frames out of 23,000)

ADMP - *atom-centered density matrix propagation*; combination of QM with MD (*Gaussian16*)

- ADMP trajectory: ~23,000 frames
- 149 frames cut from original trajectory for parameterization purposes (in average 1 frame per 154)
- Each of geometry from the short trajectory optimized (with constraints on Fe - X bonds and X-Fe-Y and X-Y-Fe angles)
- QM energies calculated with UB3LYP-D3 functional and Def2TZVP basis set

SEMINARIO[4]

- the easiest of investigated methods
- uses Hessian matrix projection for MM parameters derivation
- what do we need?
  - *.fchk file generated from *.chk file obtained after freq calculation with use of the formchk program (Gaussian)
  - xyzviewer for force constants reading

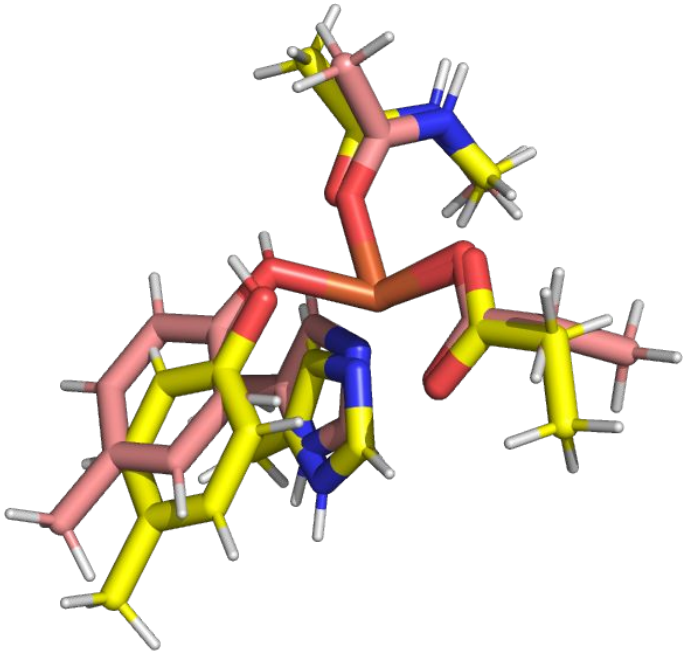


Fig. 6 Optimized geometries alignment: **QM** (salmon), **seminario** (yellow)

PARMHESS-1[5,6]

- PHF - partial hessian fitting
- what do we need?
  - python3 environment
  - Gaussian09 (local access)
  - AmberTools (resp, antechamber)
  - *.log file from freq calculations
  - *.fchk file from freq calculations
  - Gaussian MM input that contains geometry, charges, connectivity section and frcmod parameters including nonbon and VDW
    - HrmBnd1 Fe OH HO XXXXXX 121.0489

- **tsubasa.py** program from the *parmhess package* automatically generates input for **parmhess.py** program: calculates RESP, MK charges, frequencies
- **parmhess.py** generates G09 inputs for optimization: ihf, phf, fhf

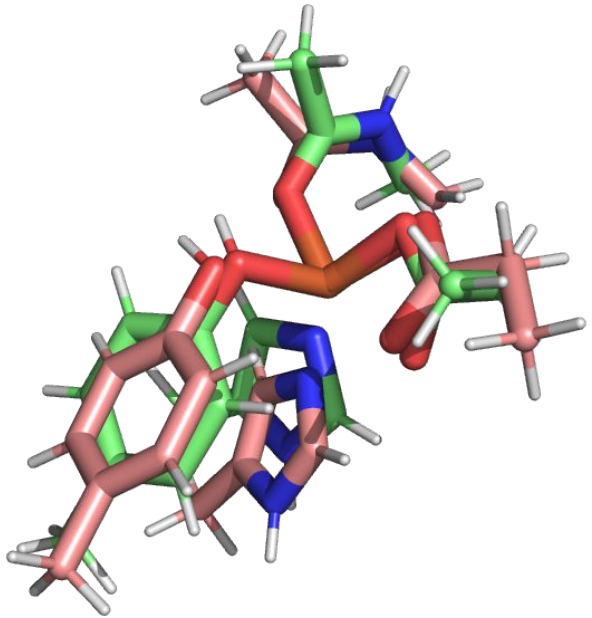


Fig. 7 Optimized geometries alignment: **QM** (salmon), **phf** (green)

PARMHESS-2[5,6]

- PHF - **katachi**
- what do we need?
  - **parmhess.py**-generated input (ihf, fhf or phf*.com)
- **katachi.py** improves input parameters and generates new G09 input for optimization

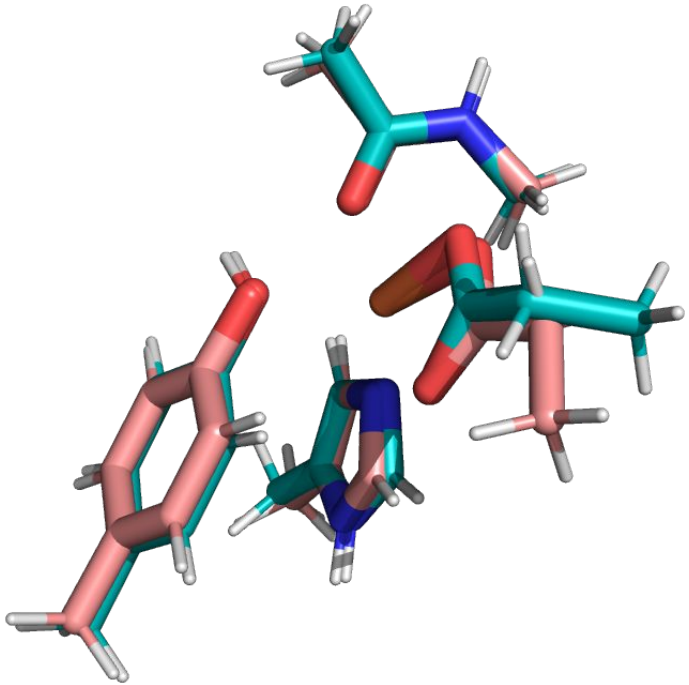


Fig. 8 Optimized geometries alignment: **QM** (salmon), **phf-katachi** (blue)

SEMINARIO-KATACHI

- **Refinement of seminario-generated parameters with katachi.py software**
- what do we need?
  - katachi.py input (as previous), this time with seminario parameters

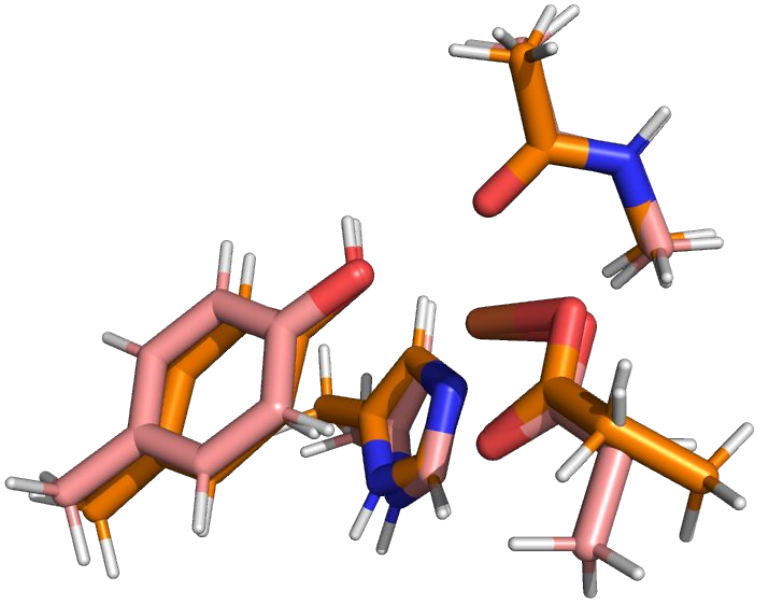


Fig. 9 Optimized geometries alignment: **QM** (salmon), **seminario-katachi** (orange)

PARAMFIT[7]

- the most time-consuming method
- available in AmberTools package
- what do we need?
  - pdb-cut model and *.in files as input for the tleap program (each amino acid described separately), with resp charges
  - trajectory (149 frames)
  - QM energies for each frame
- paramfit
  - generates Gaussian inputs (calculated on prometheus),
  - generates *.paramfit file that contains data on fitted parameters (*Fe-X, X-Fe-Y, X-Y-Fe*)
  - does parameters fitting on the basis of ADMP trajectory (149 frames long) and QM energies
  - user-friendly for tuning purposes

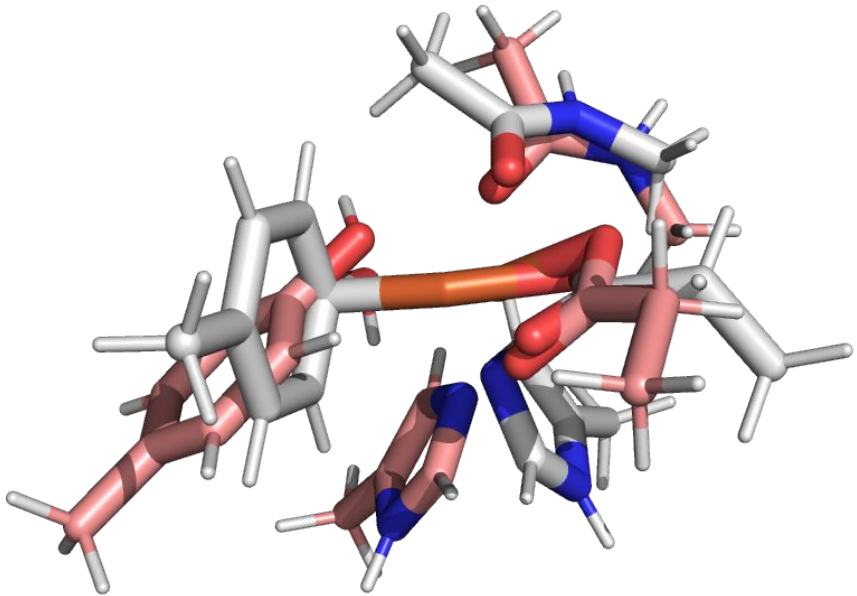


Fig. 10 Optimized geometries alignment: **QM** (salmon), **paramfit** (silver)

PARAMFIT-TUNED

- paramfit parameters with only one K and one th_{eq} changed (O2-FE-O parameters taken from the seminario method)

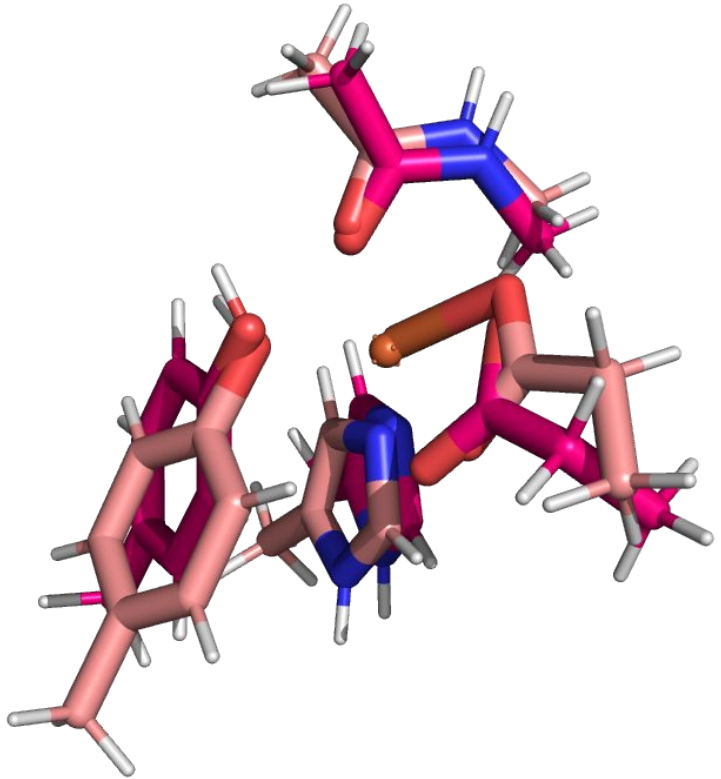
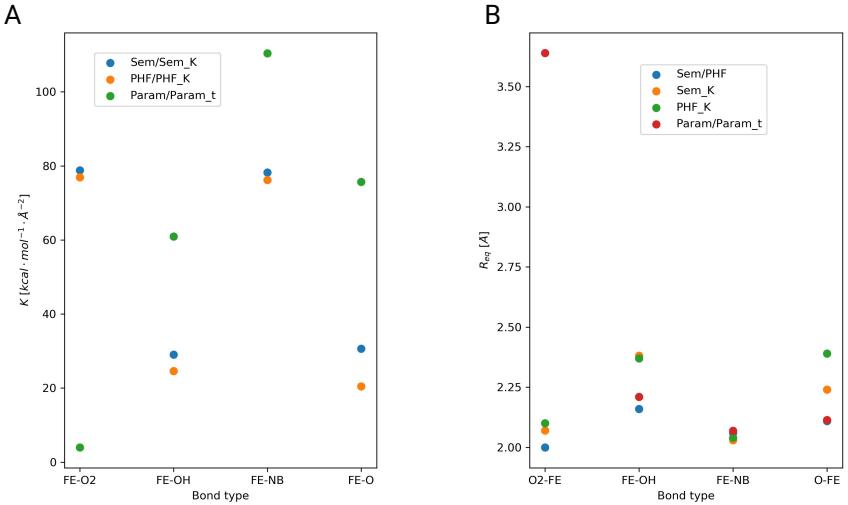


Fig. 11 Optimized geometries alignment: **QM** (salmon), **paramfit-tuned** (magenta)

PARAMETERS



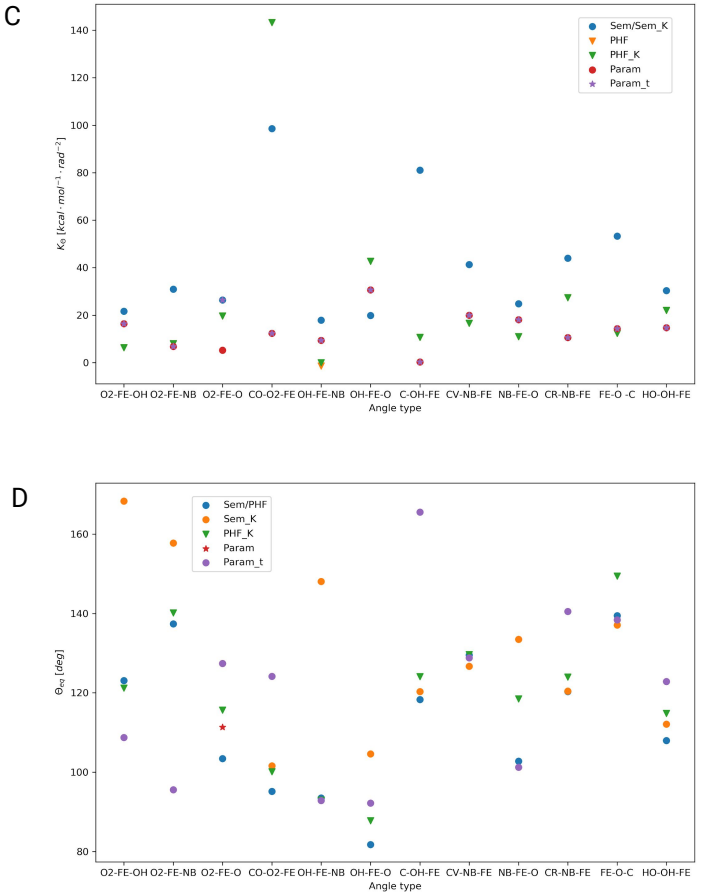


Fig. 12 Plots presenting derived parameters for each method: A) K_{bond}, B) R_{eq}, C) K_{angle}, D) Th_{eq}

Quality assessment criteria

quick sander MD simulation was run for each set of parameters in order to obtain angle/bond values distributions and calculate means

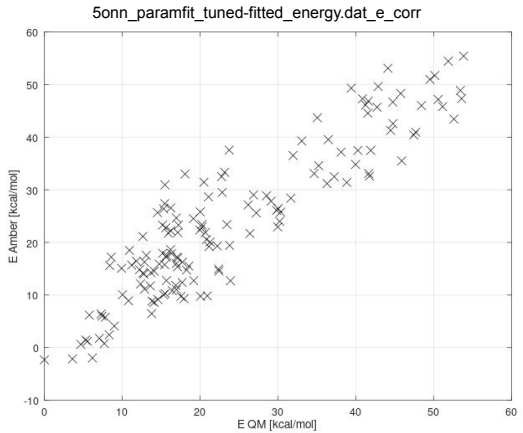
1. Convergence of the optimized geometry with the referential one (graphics) - based on differences between bond/angle values
2. QM and AMBER (fitted) energies convergence (R^2 coefficient) calculated with **paramfit**
3. Consistency of mean angle/bond values with means calculated for the **ADMP** (~23,000 frames)

Quality assessment

	SEMINARIO	PARMHESS-1	PARMHESS-2	SEMINARIO-KATACHI	PARAMFIT	PARAMFIT-TUNED
R^2	0.42	0.46	0.46	**0.3**	**0.94**	**0.83**

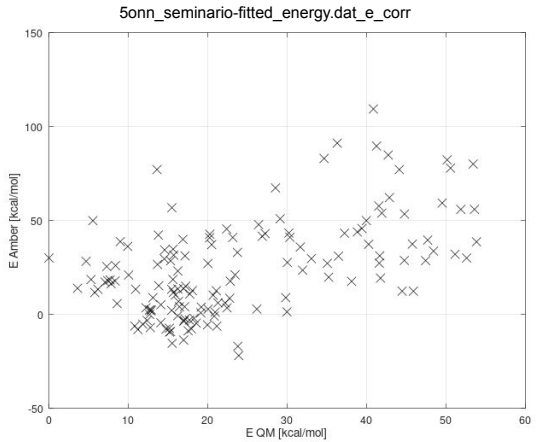
Tab. 1 Energy correlation coefficients calculated with paramfit for each method

A



$R^2 = 0.83$

B

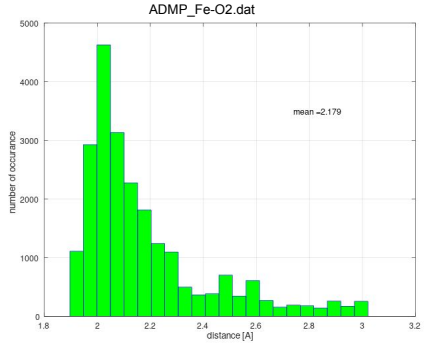


$R^2 = 0.3$

Fig. 13 Plots E_{Amber} vs E_{QM} generated for A) PARAMFIT-TUNED and B) SEMINARIO-KATACHI

Quality assessment

A



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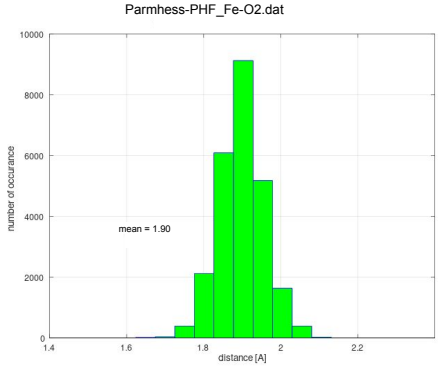


C

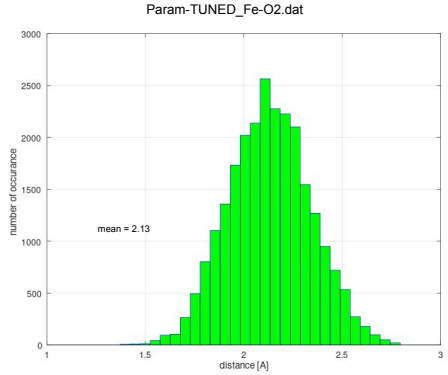
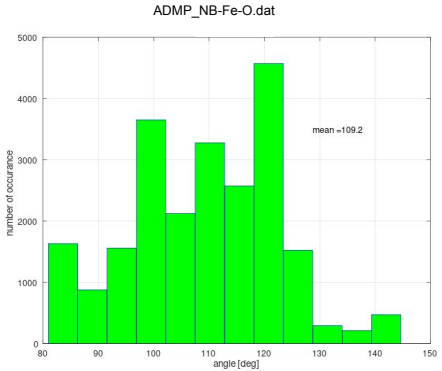
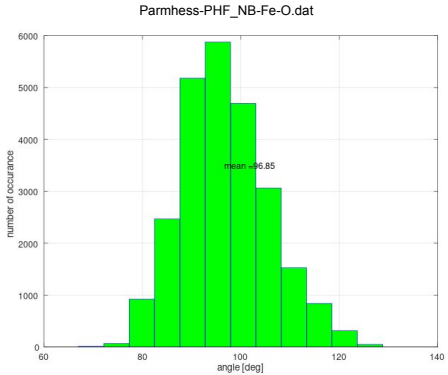


Fig. 14 Histograms for bond Fe-O2 distributions: A) ADMP B) Parmhess-PHF C) Paramfit-TUNED

A



B



C

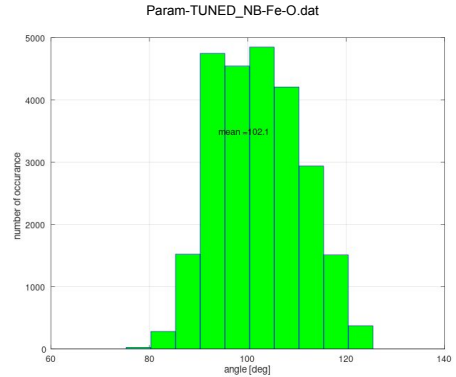


Fig. 15 Histograms for NB-Fe-O angle distributions: A) ADMP (reference) B) Parmhess-PHF C) Paramfit-TUNED

Conclusions

1. Three methods with variations were tested
2. One criterion is not enough to assess method's quality properly
3. Best optimized geometry was obtained with ***parmhess-phf*** method
4. Best correlation between fitted energy and QM energy (represented by R^2) was obtained with ***paramfit*** method
5. Best results for angle/bond means from MD were assigned to the ***paramfit_tuned*** method (smallest errors)
6. Overall, ***paramfit_tuned*** approach, where parameters describing just one angle were switch with **seminario** parameters, was chosen as the method providing the best results for investigated model

Thank you for the attention :)

Special thanks to Tomek

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Sources

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