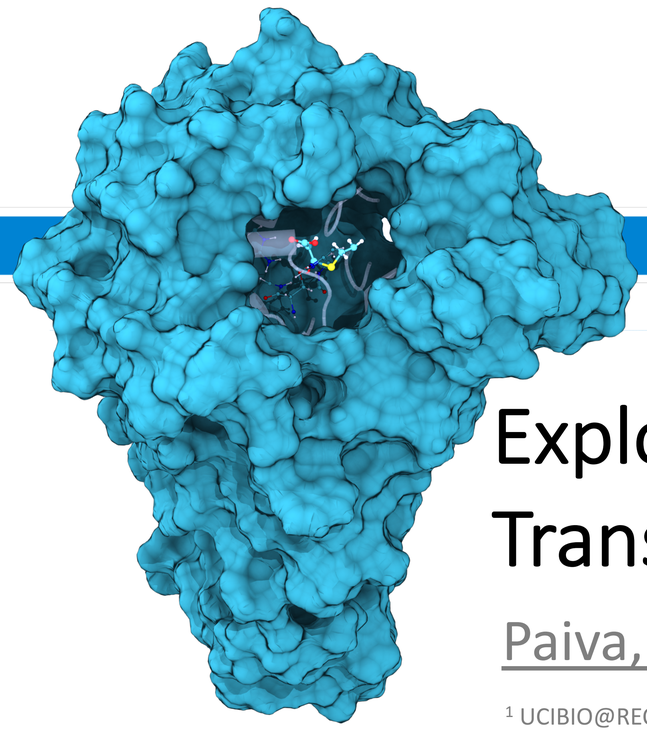

EJIBCE 2018

Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Exploring the Catalytic Mechanism of the Malonyl-Acetyl Transferase Domain of Human Fatty Acid Synthase

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INTRODUCTION

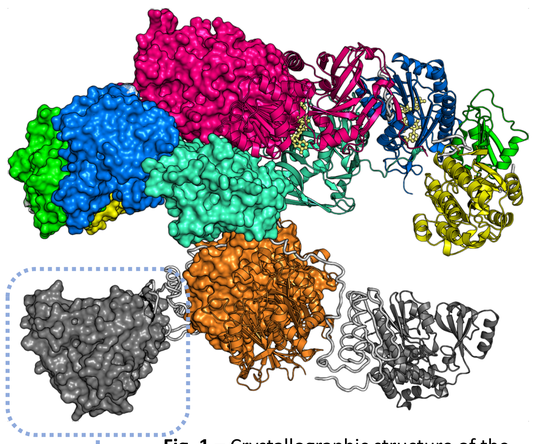


Fig. 1 – Crystallographic structure of the mammalian Fatty Acid Synthase (PDB: 2VZ8).

Malonyl-**A**cetyl **T**ransferase (MAT) Domain

Loads the initiation primer and the elongation substrate of the FA biosynthesis

- Synthesizes saturated fatty acids (FA) using acetyl-CoA and malonyl-CoA
- Seven catalytic domains: MAT, KS, KR, DH, ER, TE and ACP
- FAS gene is up-regulated in several human cancer cells
- FAS inhibitors display anti-cancer properties

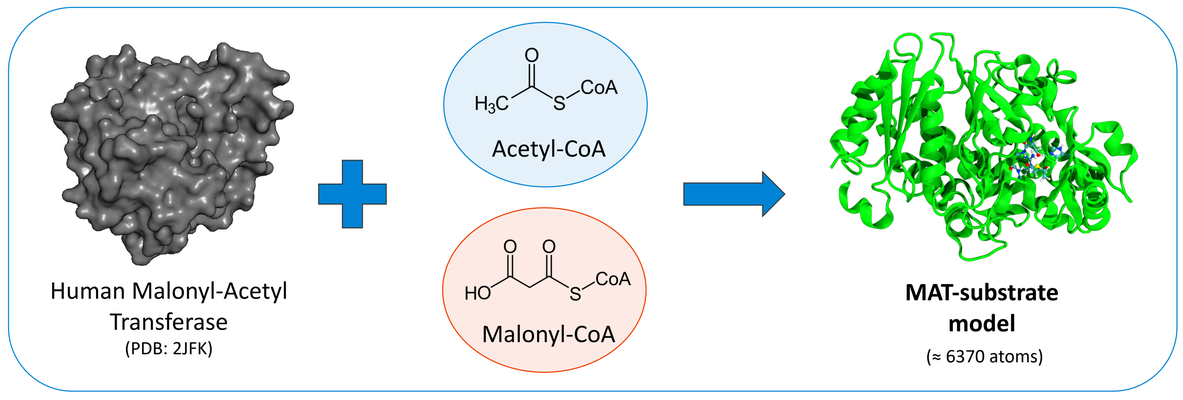
Study the catalytic mechanism of the Malonyl-Acetyl Transferase (MAT) domain

Ping-Pong mechanism

PING
MAT receives acyl moiety from CoA

PONG
MAT transfers acyl moiety to the ACP domain

METHODS



Minimization protocol
- 1. Acyl moiety minimization
- 2. H_2O minimization
- 3. H atoms minimization
- 4. Side chains minimization
- 5. Everything free
- SANDER module of AMBER 12 software

Molecular dynamics
- 500 ps (50 ps Eq. + 450 ps Prod.)
- TIP3P water model, radii 12 Å
- AMBER 12 software

ONIOM QM/MM
- QM Layer ≈ 60 atoms
- Linear-transit scans B3LYP/6-31G(d):AMBER
- Geometry optimizations B3LYP/6-31G(d):AMBER
- Frequency calculations B3LYP/6-31G(d):AMBER
- Single-Point calculations B3LYP/6-311+G(2d,2p)-D3:AMBER
- Gaussian 09 (version D) software

DLPNO-CCSD(T)
- Isolated QM Layer
- Single-Point calculations cc-pVDZ and cc-pVTZ
- Extrapolation to CBS limit
- ORCA 4.0.1.2 software

RESULTS

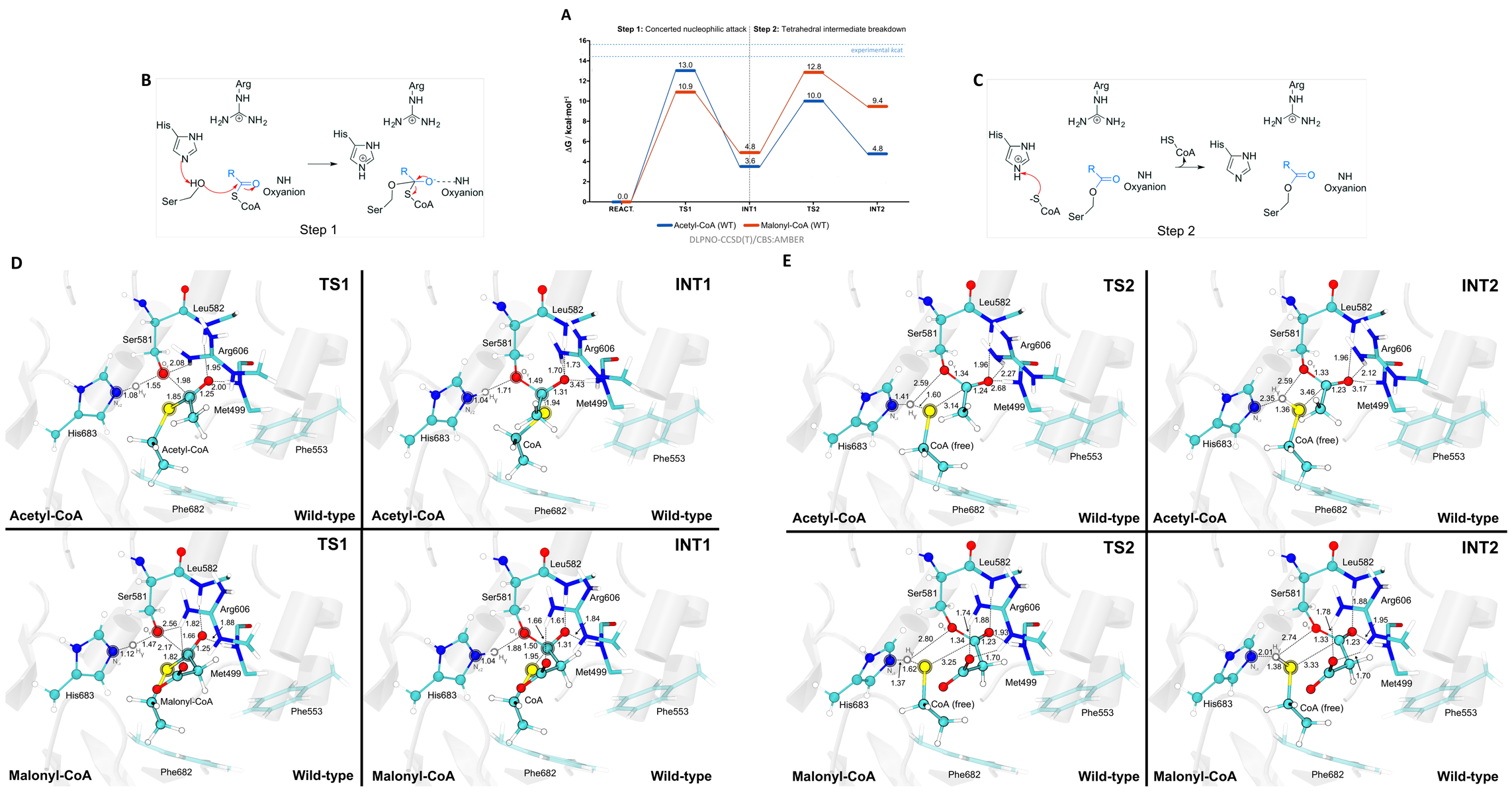


Fig. 2 – The transfer of acetyl and malonyl moieties from CoA to the catalytic Ser581 residue occurs in two consecutive catalytic steps. **A)** Free-energy profile (DLPNO-CCSD(T)/CBS:AMBER) for the acyl transfer reaction from CoA to MAT. **B)** Concerted nucleophilic attack of Ser581 on the thioester carbon (Step1). **C)** Tetrahedral intermediate breakdown (Step2). **D and E)** Optimized structures of the transition-states (TS) and intermediates (INT) of both catalytic steps; QM layer atoms represented in ball-and-stick form; values are presented in Å.

What is the energetic contribution of the oxyanion hole for the concerted nucleophilic attack?

Replacement
(Met499 and Leu582)
NH group → CH_2 group

Oxyanion hole lowers the Step 1 activation energy by:
MAT-acetyl-CoA: 6.7 kcal·mol^{-1}
MAT-malonyl-CoA: 3.6 kcal·mol^{-1}

(Literature (trypsin, subtilisin, KSI): 4-6 kcal·mol^{-1})

CONCLUSIONS

- Acyl transfer from CoA to MAT occurs in two steps:
  - Step 1: Concerted nucleophilic attack, centered on a Ser-His catalytic dyad
  - Step 2: Tetrahedral intermediate breakdown
- Arg606 and the hydrophobic pocket are important for positioning the malonyl and acetyl moieties, respectively
- The backbone amines of Met499 and Leu582 form an oxyanion hole that stabilizes the TS1 and INT1 for MAT-acetyl-CoA and MAT-malonyl-CoA complexes

REFERENCES:
- T. Maier, M. Leibundgut, N. Ban, *Science* **2008**, *321*, 1315-1322
- P. R. Pandey, W. Liu, F. Xing, K. Fukuda, K. Watabe, *Recent Pat. Anticancer Drug Discov.* **2012**, *7*, 185-197
- C. Riplinger, F. Neese, *J. Chem. Phys.* **2013**, *138*, 034106
- S. Smith, S. C. Tsai, *Nat. Prod. Rep.* 2007, 24, 1041-1072

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