The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Molecular modelling studies on PEA hydrolysis by the enzyme *N*-acylethanolamine acid amidase.

Laura Scalvini[1,*], Andrea Ghidini[1], Alessio Lodola[1], Donatella Callegari[1], Silvia Rivara[1], Daniele Piomelli[2,3,4] and Marco Mor[1]

[1] Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli Studi di Parma, Parco Area delle Scienze 27A I-43124 Parma, Italy;
[2,3,4] Department of Anatomy and Neurobiology, Department of Pharmaceutical Sciences, and Department of Biological Chemistry and Molecular Biology, University of California, Irvine, California 92697-4625, United States.

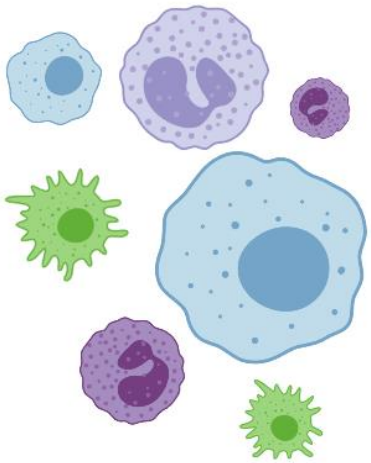
* Corresponding author: laura.scalvini@unipr.it

Abstract:
N-acylethanolamine acid amidase (NAAA) is an N-terminal cysteine hydrolase involved in the hydrolytic inactivation of the lipid mediator palmitoylethanolamide (PEA), showing an optimal activity in acidic conditions. Starting from the X-ray structure of the human NAAA we investigated the mechanism of the hydrolytic inactivation of PEA operated by NAAA, using a multiscale approach in which we combined molecular dynamics and mechanical/molecular mechanics (QM/MM) simulations coupled with enhanced sampling. Our simulations pointed out the critical role of the proton configuration of the catalytic residue, Cys126, and of the acid residues situated in the close proximity to the active site in preserving the architecture of the catalytic site. Starting from a stable Michaelis complex, we reconstructed the free energy profile of NAAA acylation and deacylation occurring during the PEA hydrolysis. Our results outlined the acylation as the rate-limiting step of the entire reaction, in which Cys126 acts as an acid, able to protonate the leaving group, and as a nucleophile, giving a nucleophilic attack on the substrate carbonyl carbon. As supported by kinetic experiments, in which we demonstrated that NAAA can efficiently hydrolyze palmitoyl methyl amide (PMA), the ethanol portion of PEA does not play an indispensable role in the reaction.

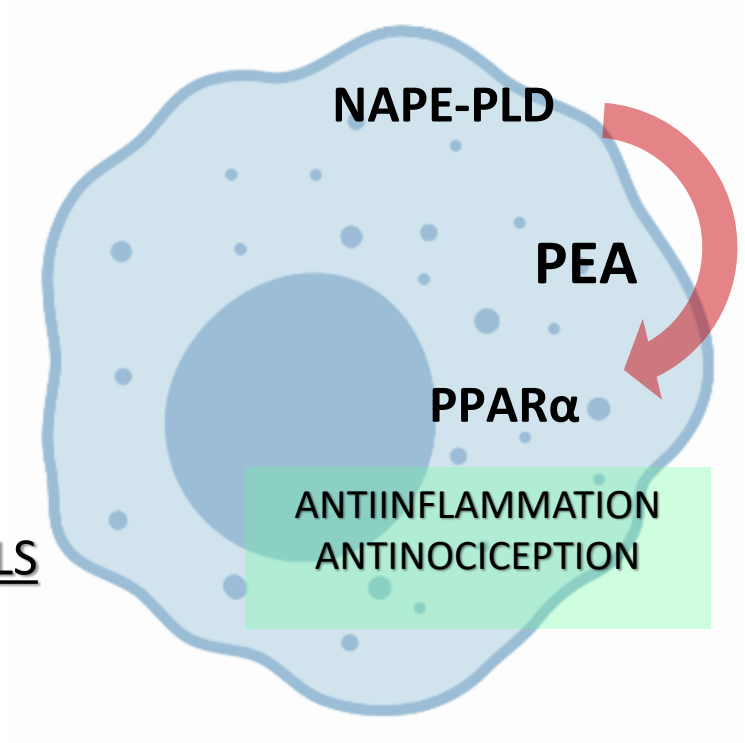
Keywords: cysteine, free-energy surface, hydrolysis, NAAA, palmitoylethanolamide, QM/MM.

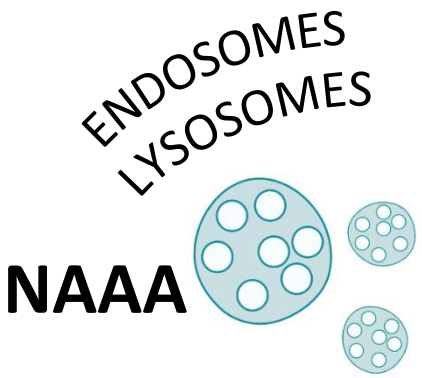
Introduction

N-acylethanolamine acid amidase (NAAA) is a lysosomal hydrolase that regulates the levels of palmitoylethanolamide (PEA), which suppresses inflammation and nociception



HOST DEFENCE CELLS
MICROGLIA
MACROPHAGES
LYMPHOCYTES





Piomelli *et al.* **2014** *Nat.Neurosci.* 17,164-174
Piomelli *et al.* **2020** *J. Med. Chem.* 63,7475-7490

Introduction

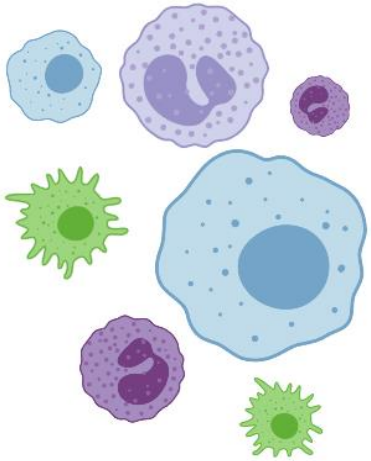
INFLAMMATORY SIGNALS

↓

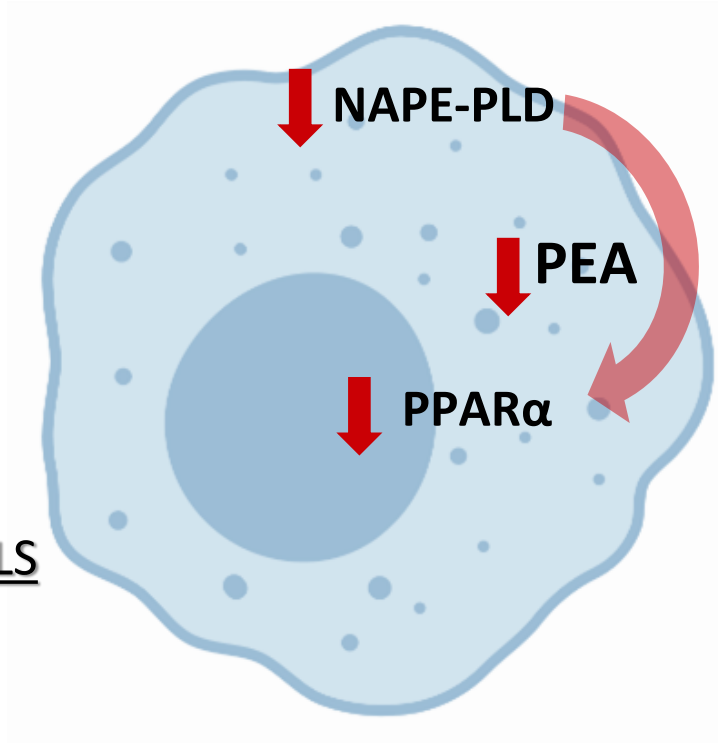
PEA LEVELS REDUCTION

↓

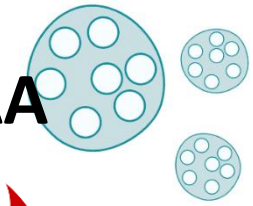
INFLAMMATION PROGRESS
NOCICEPTION TRESHOLD
DECREASE



HOST DEFENCE CELLS
MICROGLIA
MACROPHAGES
LYMPHOCYTES



ENDOSOMES
LYSOSOMES

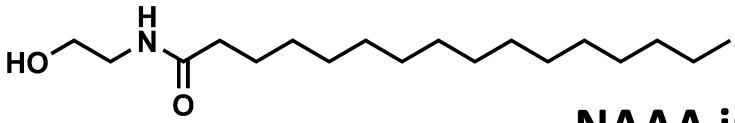


↑ NAAA

Piomelli *et al.* **2014** *Nat.Neurosci.* 17,164-174
Piomelli *et al.* **2020** *J. Med. Chem.* 63,7475-7490

Introduction

PEA

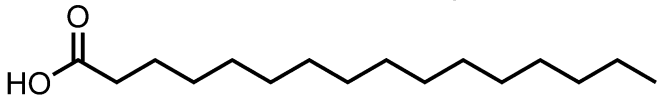


NAAA inhibitors

PEA signaling normalization in injured/inflamed tissues

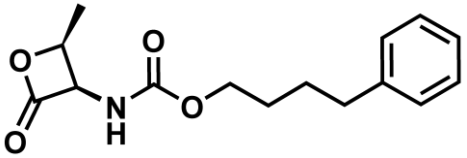
Solorzano *et al.* **2009** *PNAS* 49, 20966-20971

NAAA



palmitic acid

URB913



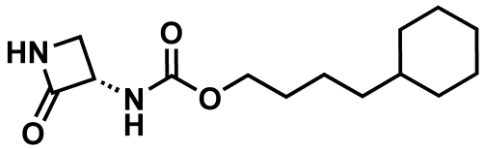
DNFB-induced allergic dermatitis

Carrageenan-induced leukocyte infiltration

Sasso *et al.* **2018** *J.Invest.Dermatol.* 138, 562-569

Solorzano *et al.* **2010** *J.Med.Chem* 53, 5770-5781

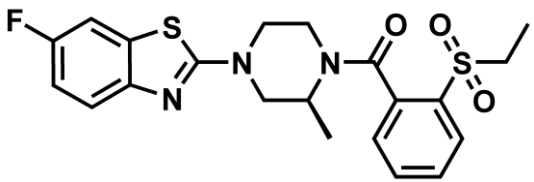
ARN726



Carrageenan-induced lung inflammation

Ribeiro *et al.* **2015** *ACS Chem.Biol.* 10, 1838-1846

ARN19702

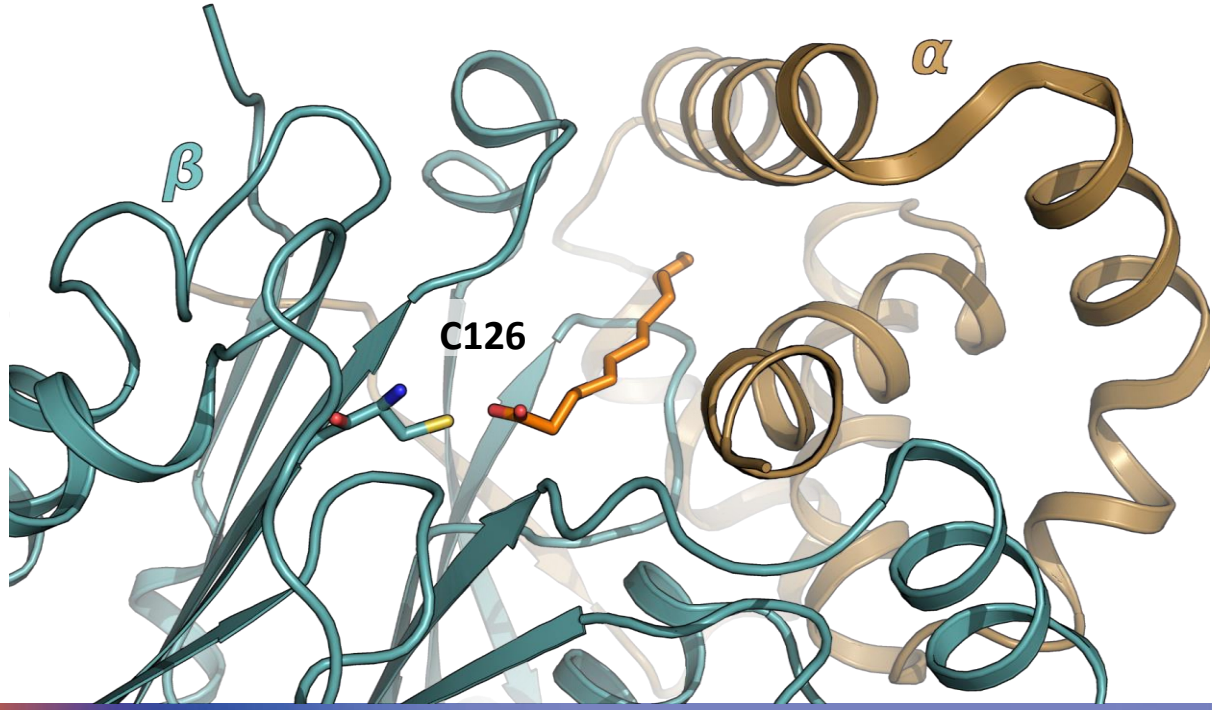


Multiple sclerosis

Migliore *et al.* **2015** *Angew.Chem.Int.Ed.Engl.* 55, 11193-11197

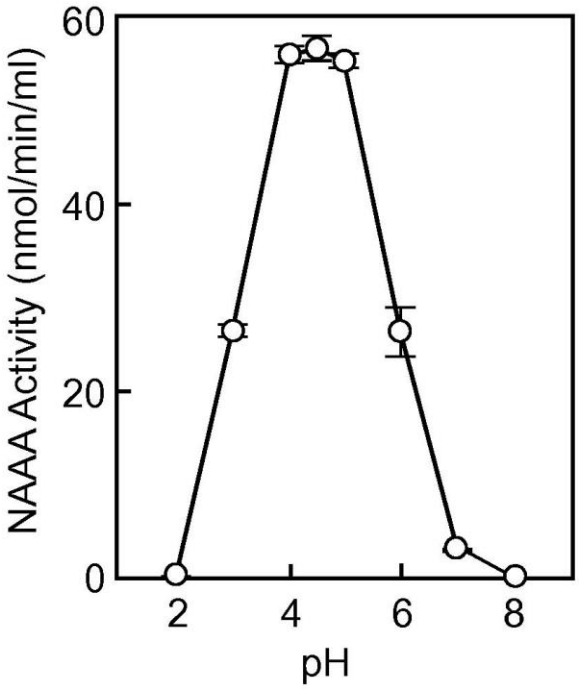
Introduction

- NTN hydrolase
  - →Autocleavage in acidic conditions generates the α and β subunits
  - →The binding site is at the interface between the α and β subunits
- Catalytic site
  - →C126 is the N terminal catalytic residue



Introduction

- NAAA has a sharp pH-activity profile, with a maximum at pH 4.5
  - Wang *et al.* **2008** *Biochim.Biophys.Acta* 1781, 710-717

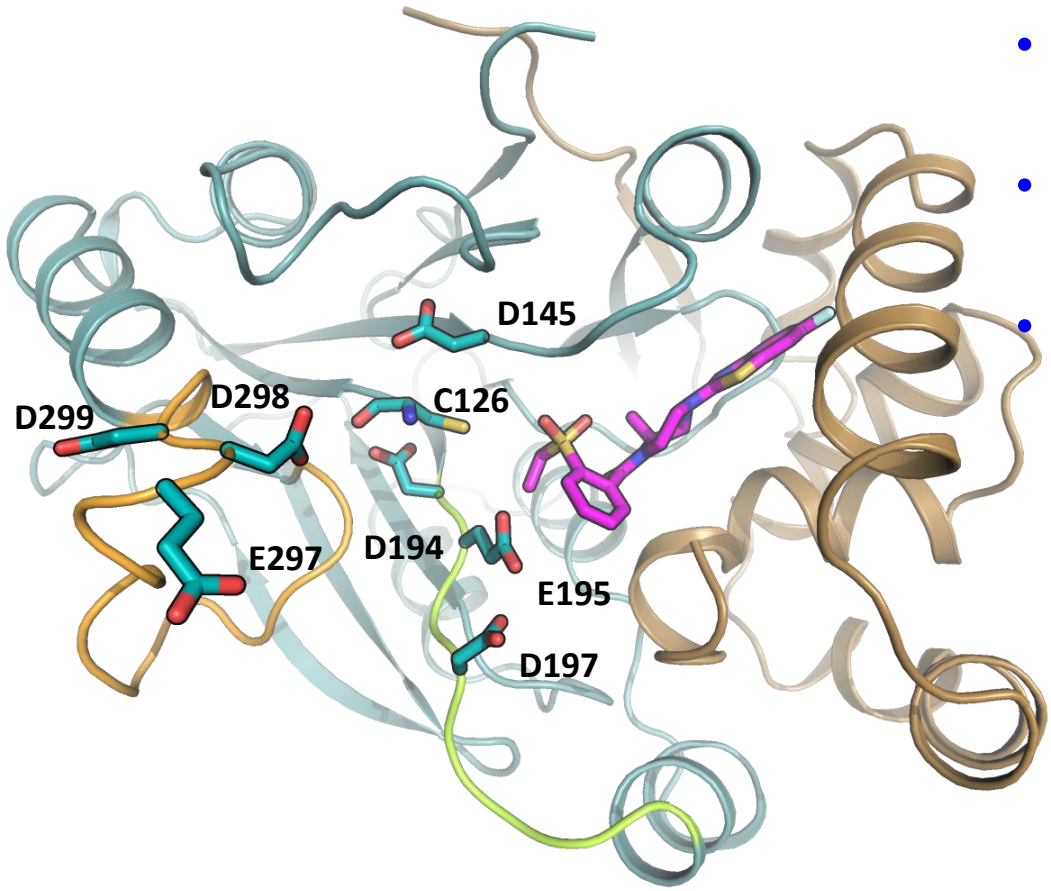


→ The dynamics of modelled NAAA depends on the protonic state of key ionizable centers

→ Issues

- Non-trivial assignment of the protonation state of
  - the catalytic residue (terminal amino group and thiol group of Cys126)
  - carboxylic groups surrounding the active site

Results and discussion



Effect of the protonic configuration on NAAA stability *via* MM studies

- Xray of hNAAA in complex with ARN19702
- Supervised assignment of the protonic configuration
- Configurations with different protonic states ($COOH/COO^-$, SH/S^-, NH_3^+/NH_2) were sampled

→C126 modelled as thiolate and with the neutral amino group/D145 modelled in its neutral form

→stable NAAA-inhibitor complex

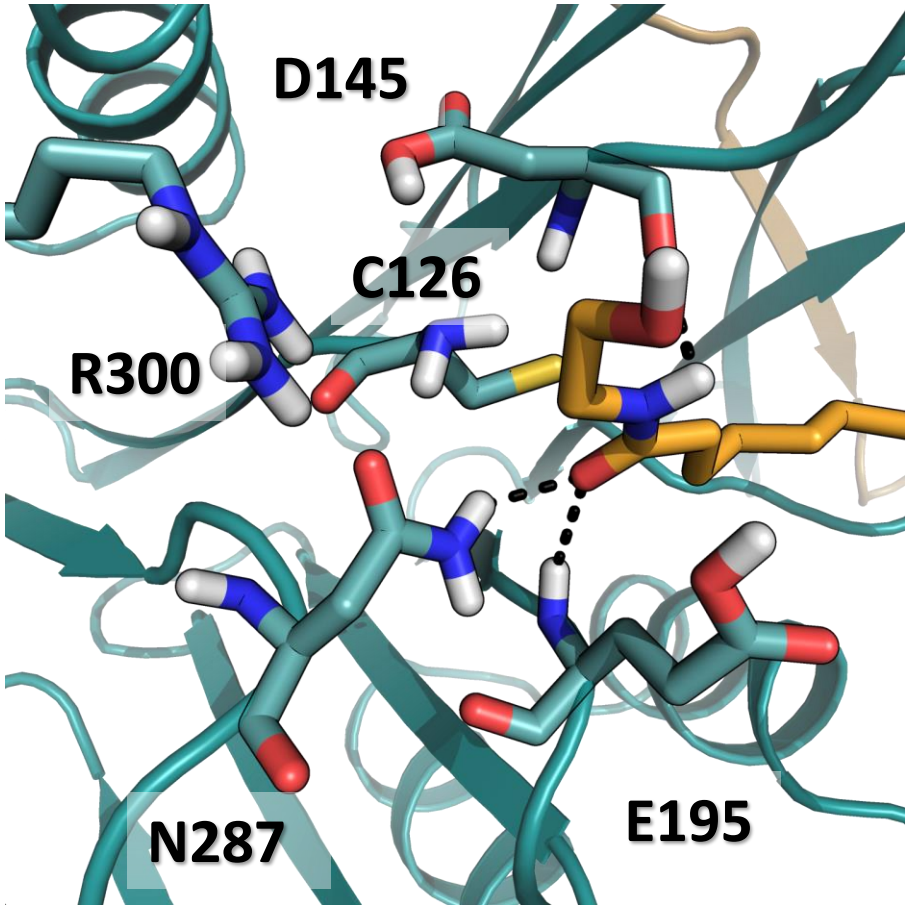
Scalvini *et al*. **2020** *ACS Catalysis* 10, 11797-11813

Results and discussion

QM/MM studies of the model of hNAAA-PEA complex

- Evaluation of proton-distribution equilibrium in presence of the substrate (PEA)
- Study of the mechanism of PEA hydrolysis

  → QM/MM studies at the SCC-DFTB3/AMBER level of theory



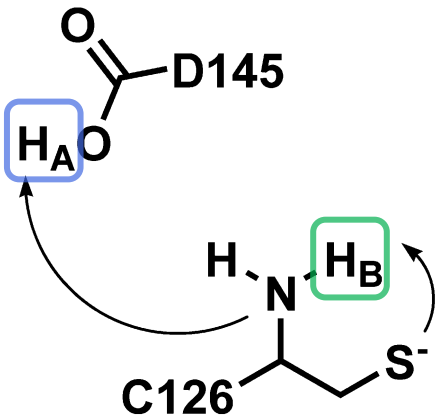
Scalvini *et al*. **2020** *ACS Catalysis* 10, 11797-11813

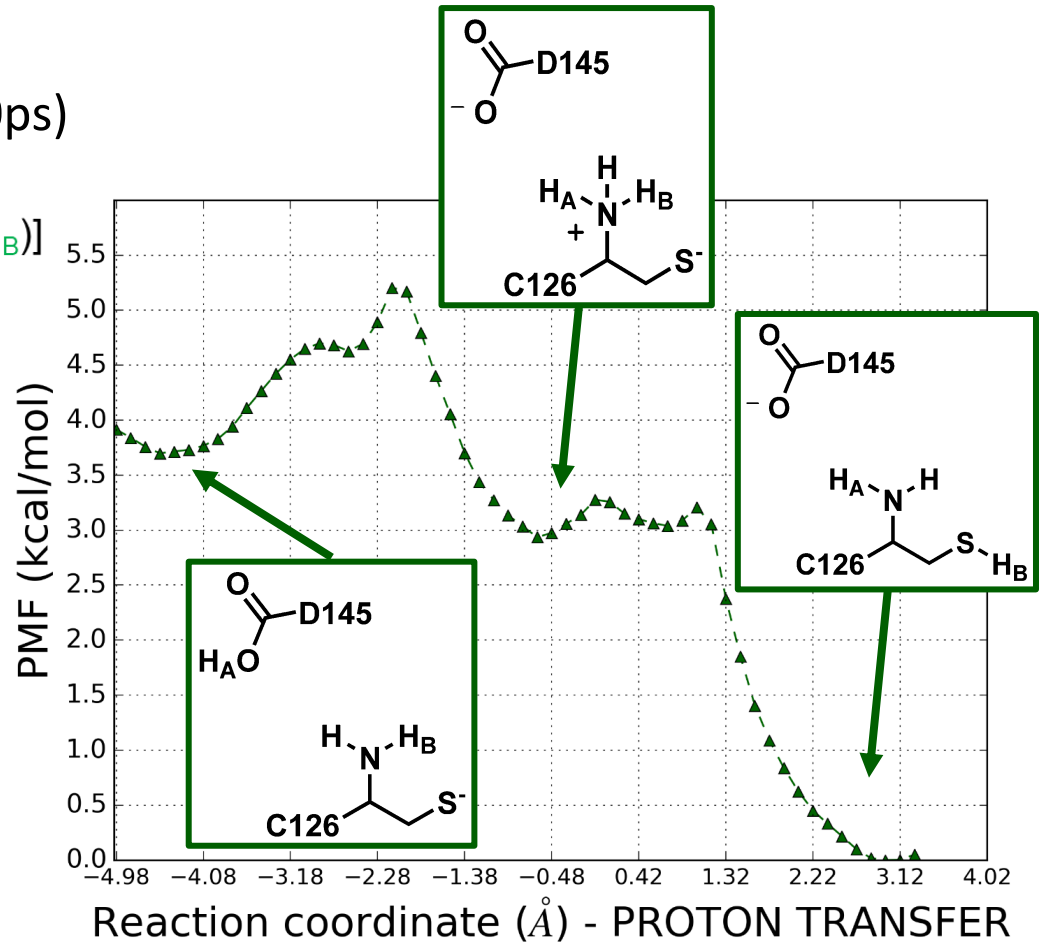
Results and discussion

Equilibrium of the proton distribution *via* QM/MM studies for NAAA-PEA complex

- Proton transfer among D145-C126 ionizable centers for the Michaelis complex (NAAA-PEA)
- Umbrella Sampling simulations (350ps)

$$[(O_{D145} - H_A) - (N_{C126} - H_A) + (N_{C126} - H_B) - (S_{C126} - H_B)]$$

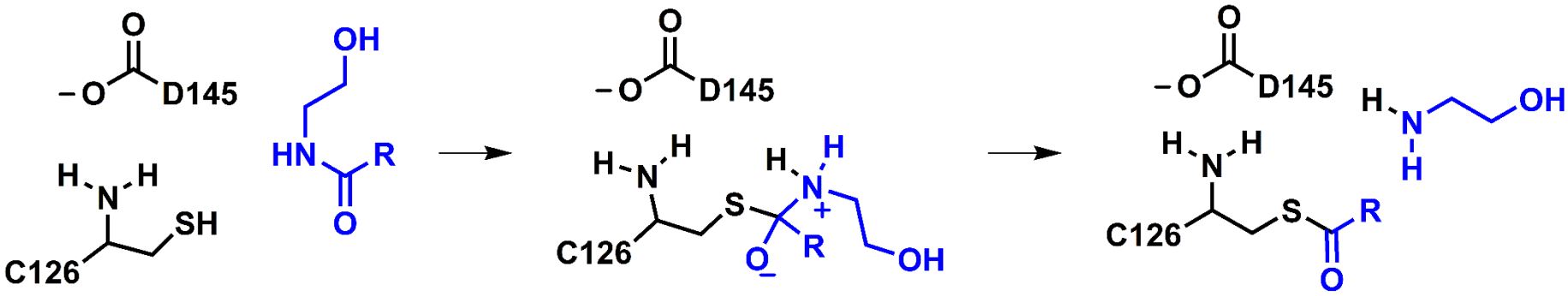




Scalvini *et al*. **2020** *ACS Catalysis* 10, 11797-11813

Results and discussion

Mechanism of PEA hydrolysis – *ACYLATION STEP*



- 2D-Umbrella sampling simulations coordinates (60 ps)

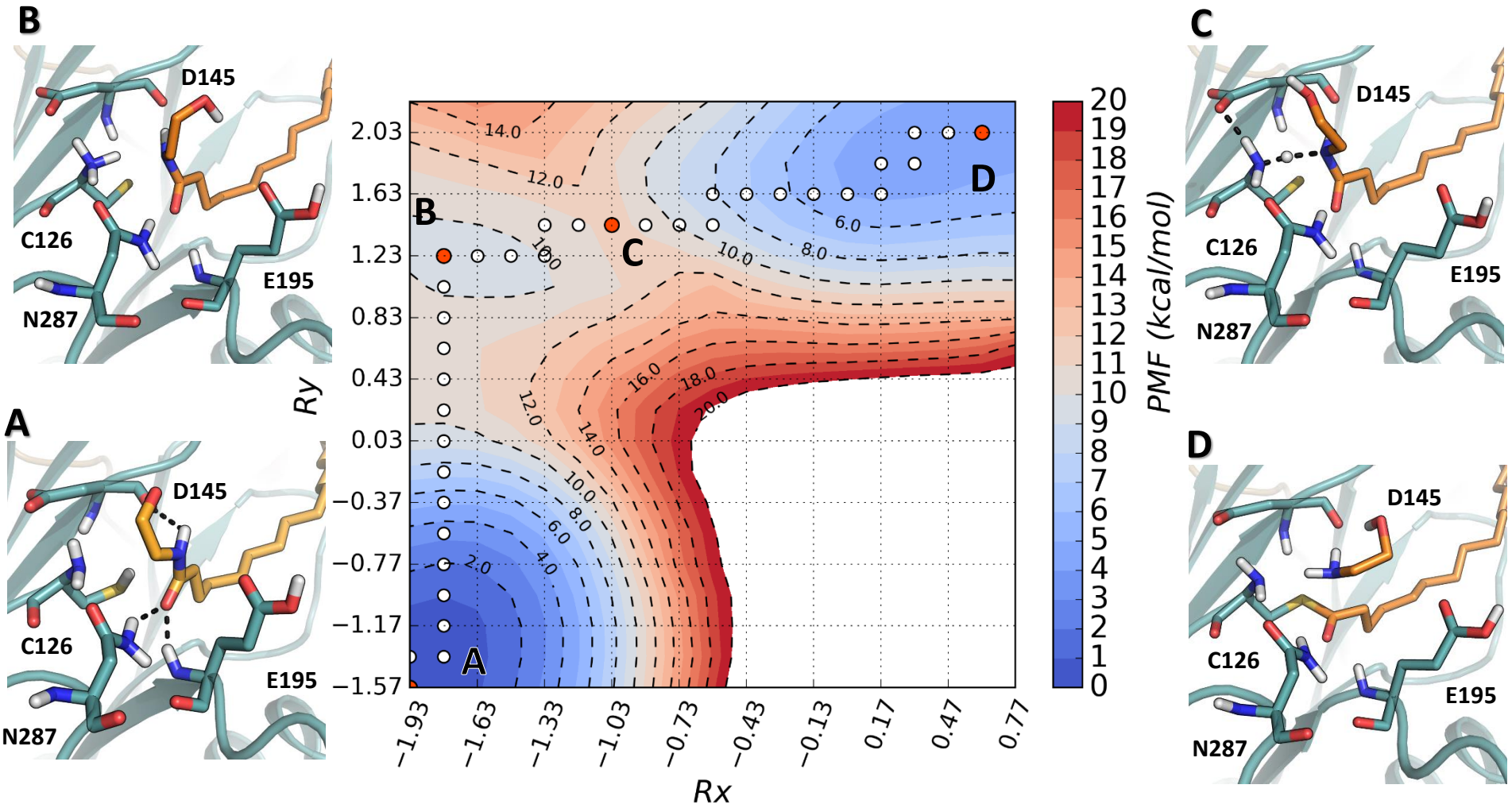
  X: $[(C_{PEA} - N_{PEA}) – (C_{PEA} - S_{C126})]$

  Y: $[(S_{C126} - H_{C126}) – (N_{PEA} - H_{C126})]$

Scalvini *et al*. **2020** *ACS Catalysis* 10, 11797-11813

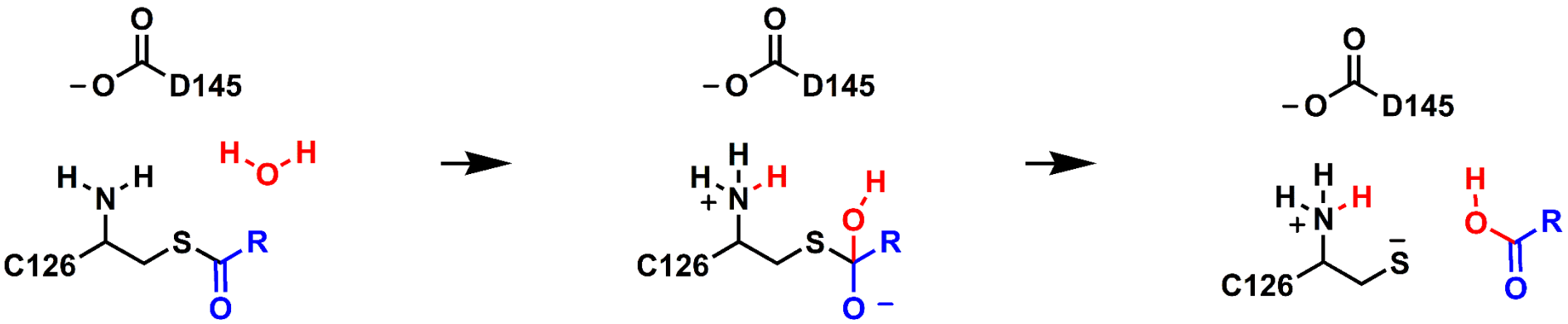
Results and discussion

Free-energy surface for the acylation step



Results and discussion

Mechanism of PEA hydrolysis – *DEACYLATION STEP*



- 2D-Umbrella sampling simulations coordinates (60 ps)

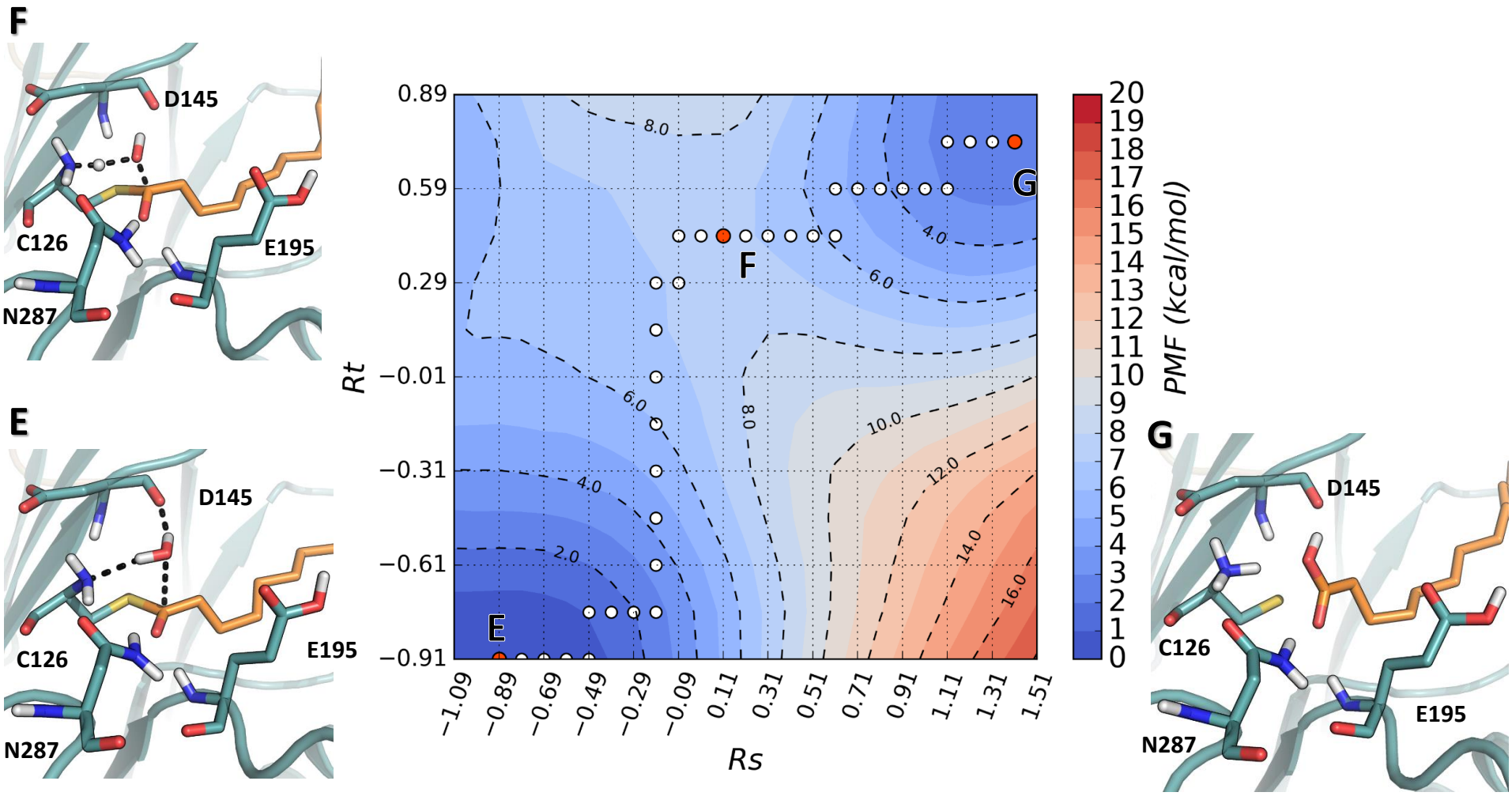
  X: $[(C_{PEA} - S_{C126}) – (O_{WAT} - C_{PEA})]$

  Y: $[(O_{WAT} - H_{WAT}) – (H_{WAT} - N_{C126})]$

Scalvini *et al*. **2020** *ACS Catalysis* 10, 11797-11813

Results and discussion

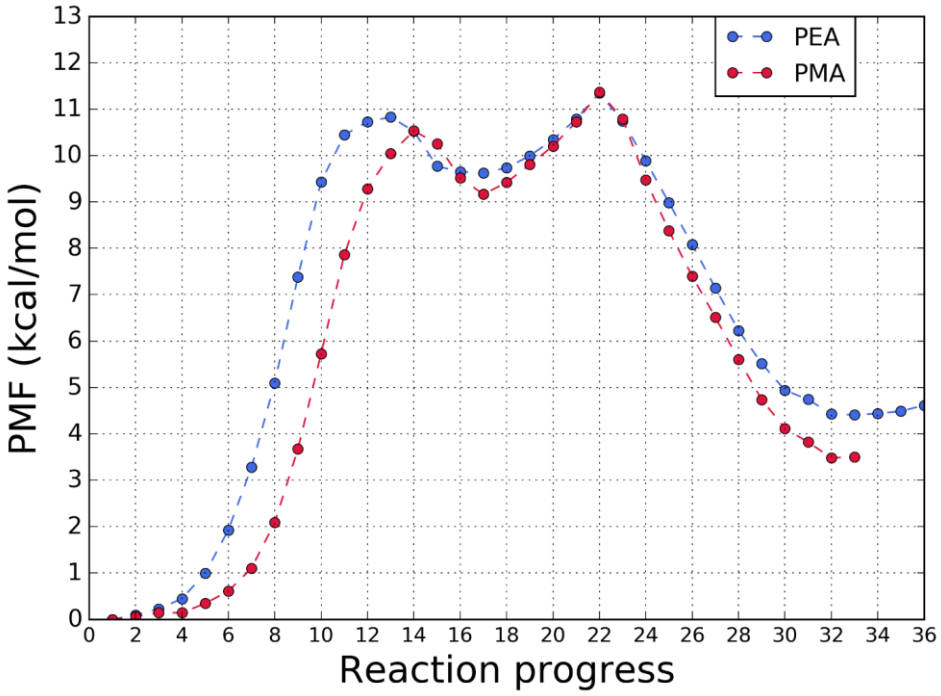
Free-energy surface for the deacylation step



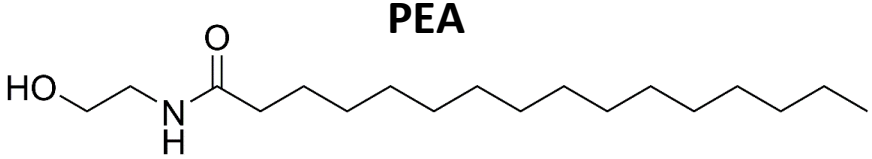
Results and discussion

Investigation of the role of the amine leaving group

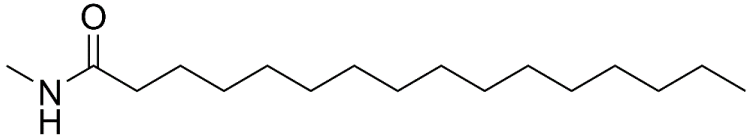
The high similarity of free-energy profile for NAAA acylation from PEA and PMA suggests that the ethanol moiety has a marginal role in catalysis

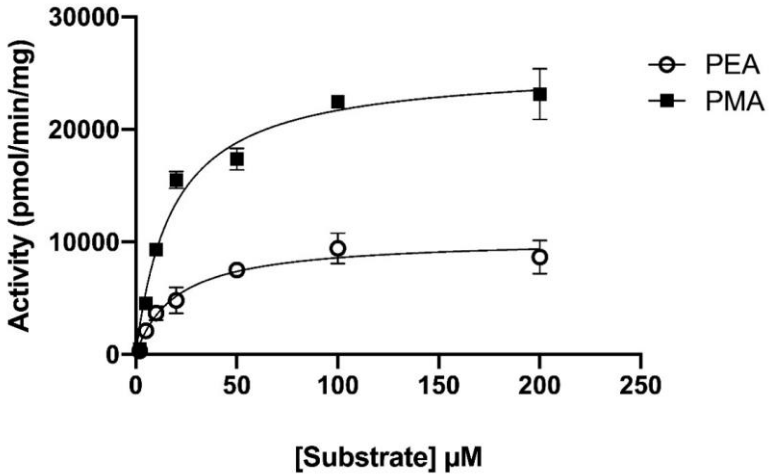


PEA



PMA



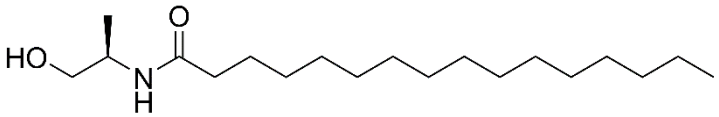


Scalvini *et al*. **2020** *ACS Catalysis* 10, 11797-11813

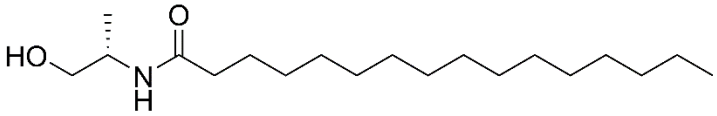
Results and discussion

Investigation of the role of the amine leaving group

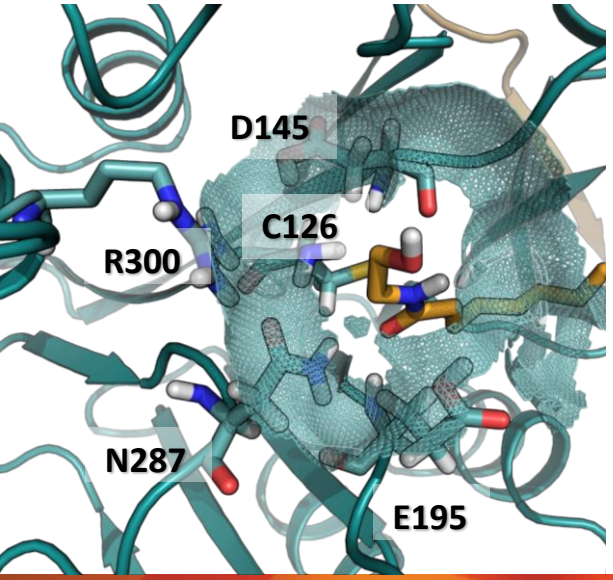
R-methyl



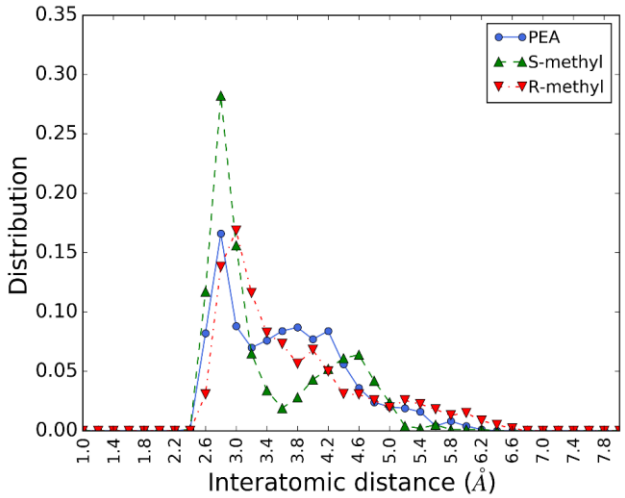
S-methyl



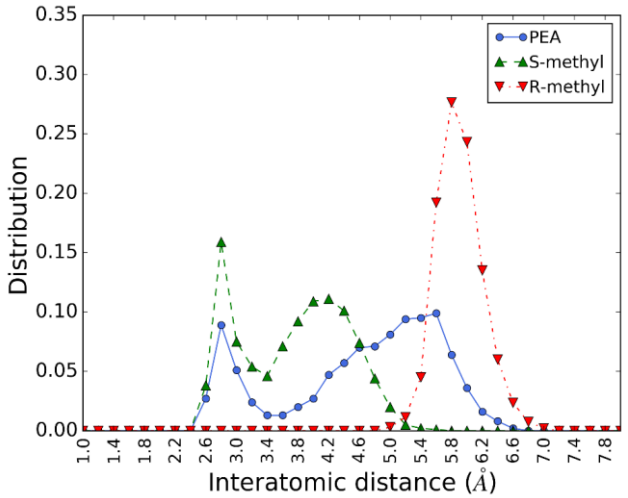
- MD simulations of NAAA in complex with PEA, *S*- and *R*-methylPEA
  - Polar interactions between the hydroxyl group and D145 are more frequent for the *S*-methyl derivative than PEA and *R*-methyl PEA
  - An additional polar contact with the terminal amino group of C126 is observed for PEA and *S*-methyl PEA



OH/D145 side chain



OH/C126 amino group

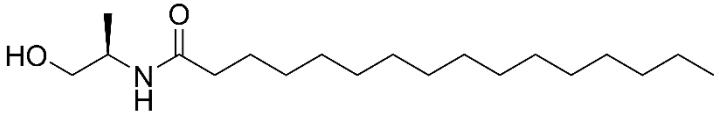


Ghidini *et al*. **2021** *J. Enz. Inhib. Med. Chem.* 36, 1411-1423

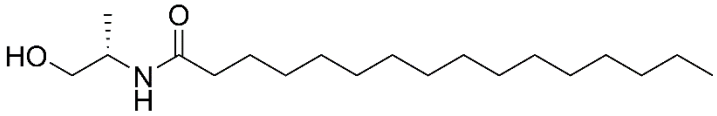
Results and discussion

Investigation of the role of the amine leaving group

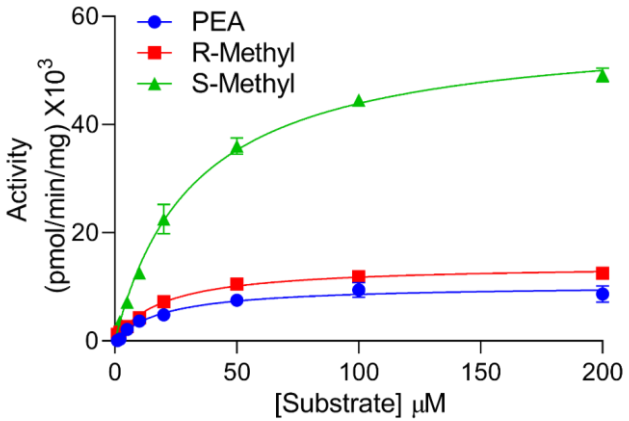
R-methyl



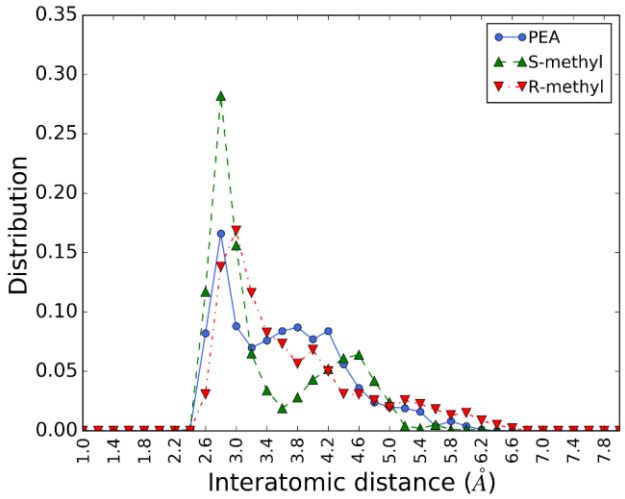
S-methyl



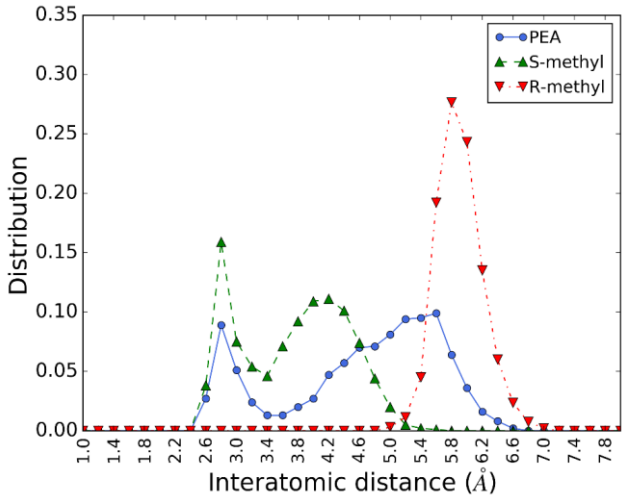
- MD simulation results mirror biochemical experiments
- relatively bulky polar groups can be accommodated within the entrance of NAAA active site



OH/D145 side chain



OH/C126 amino group



Ghidini *et al*. **2021** *J. Enz. Inhib. Med. Chem.* 36, 1411-1423

Conclusions

- A fine regulation of the protonation state of amino acids within the active site is fundamental for the conformational stability of the enzyme
  - MD simulations allowed the identification of an optimal configuration of Cys126 and of close ionizable groups

- QM/MM studies support an acid-catalyzed acylation mechanism for PEA hydrolysis
  - Energetics of the modelled reaction is consistent with catalytic activity

- Our model explain the role of the leaving group and provide a rationalization for the effect of different substituents at ethanolamine chain