SMALL-MOLECULE MODULATION OF HUMAN NAPE-PLD SIGNALING

Nanosciences – 3rd year PhD annual exam

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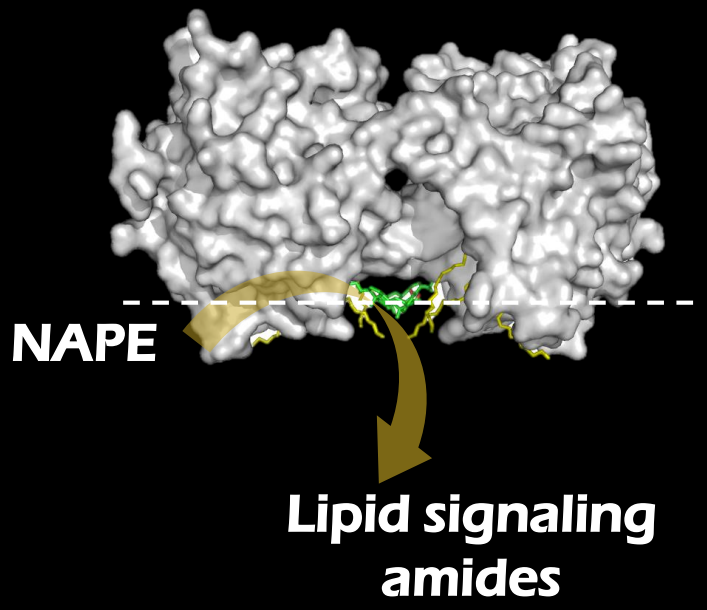
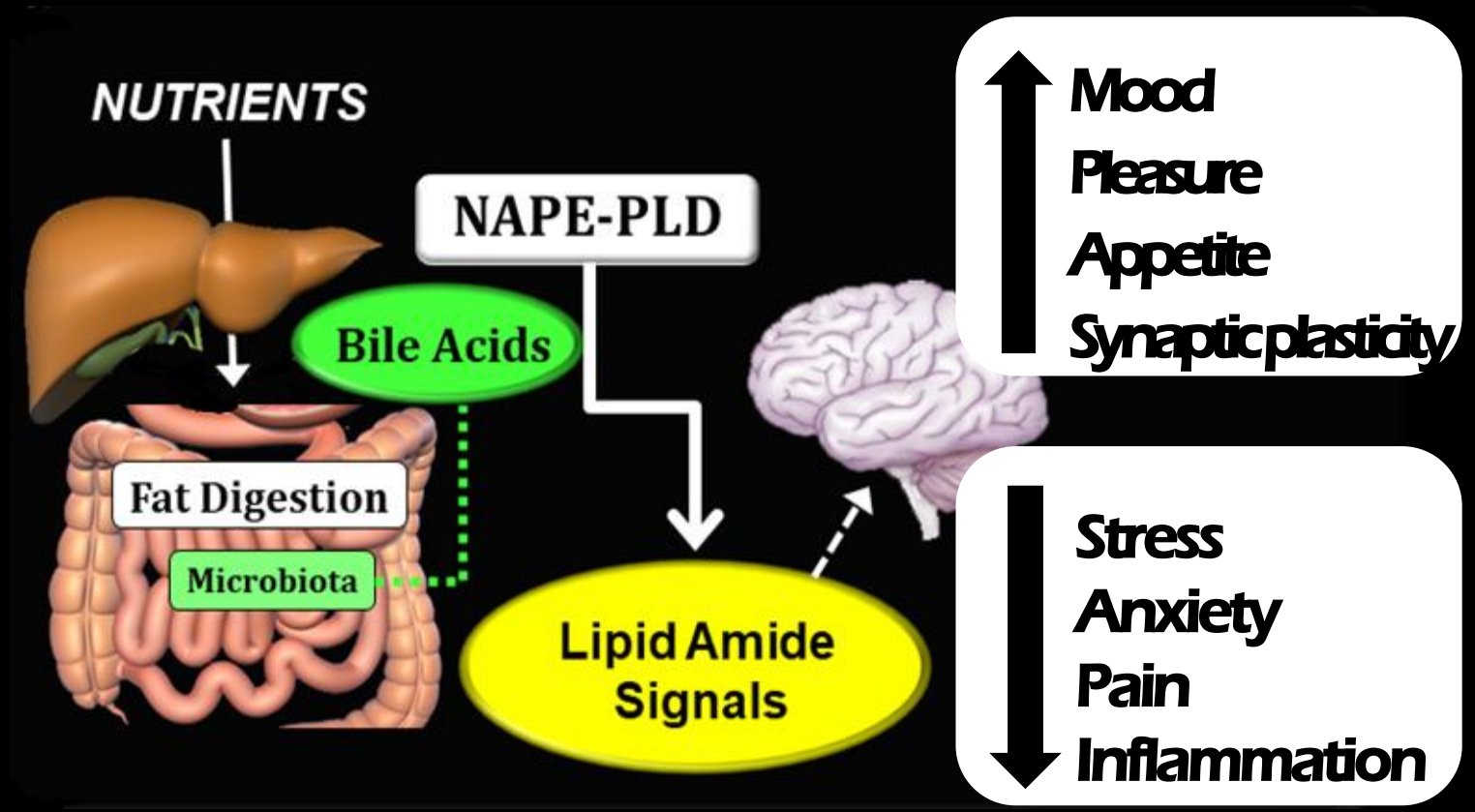
Pisa, 20/10/2020

BIOACTIVE LIPID AMIDES

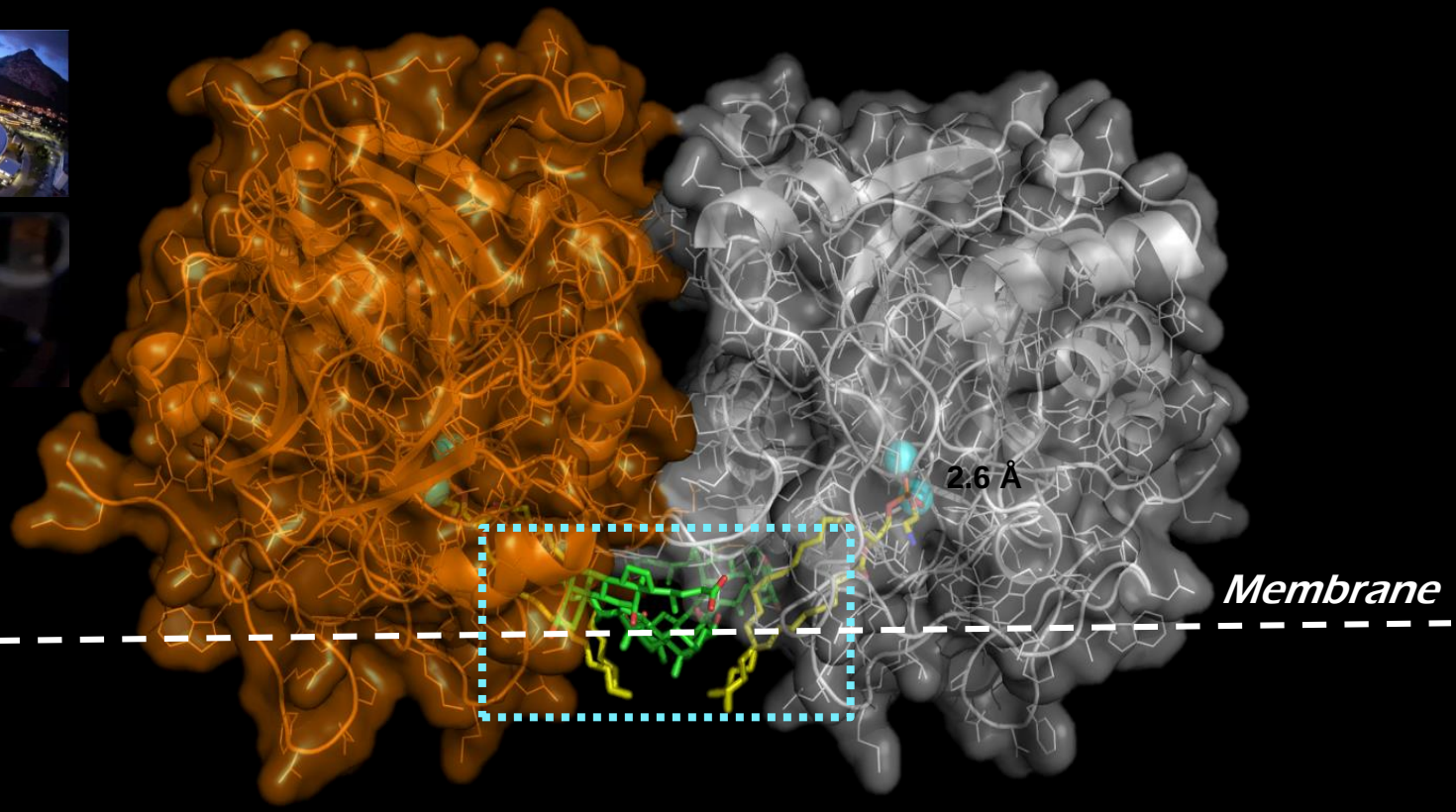


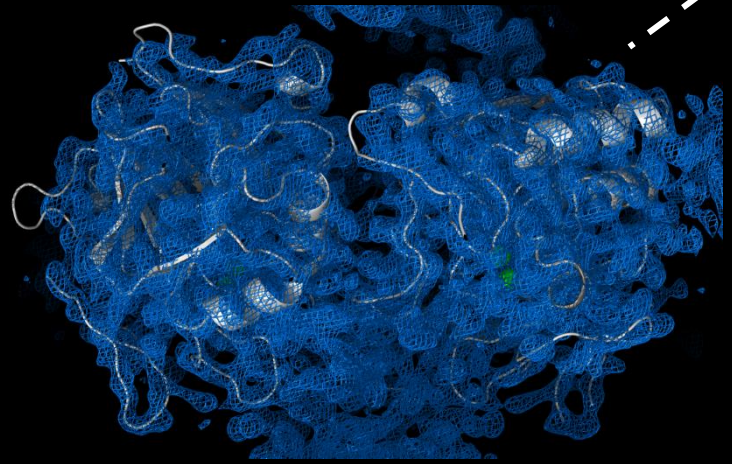


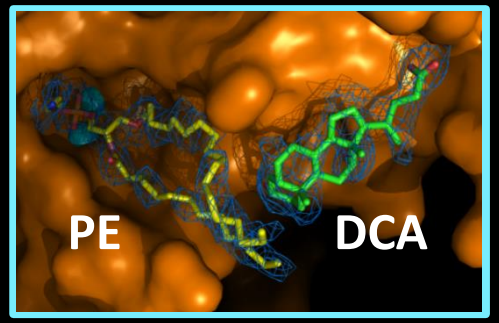
Modulation for potential therapeutic application

STRUCTURE OF HUMAN MEMBRANE NAPE-PLD

ELETTRA XRD1
ESRF beamline ID23-2

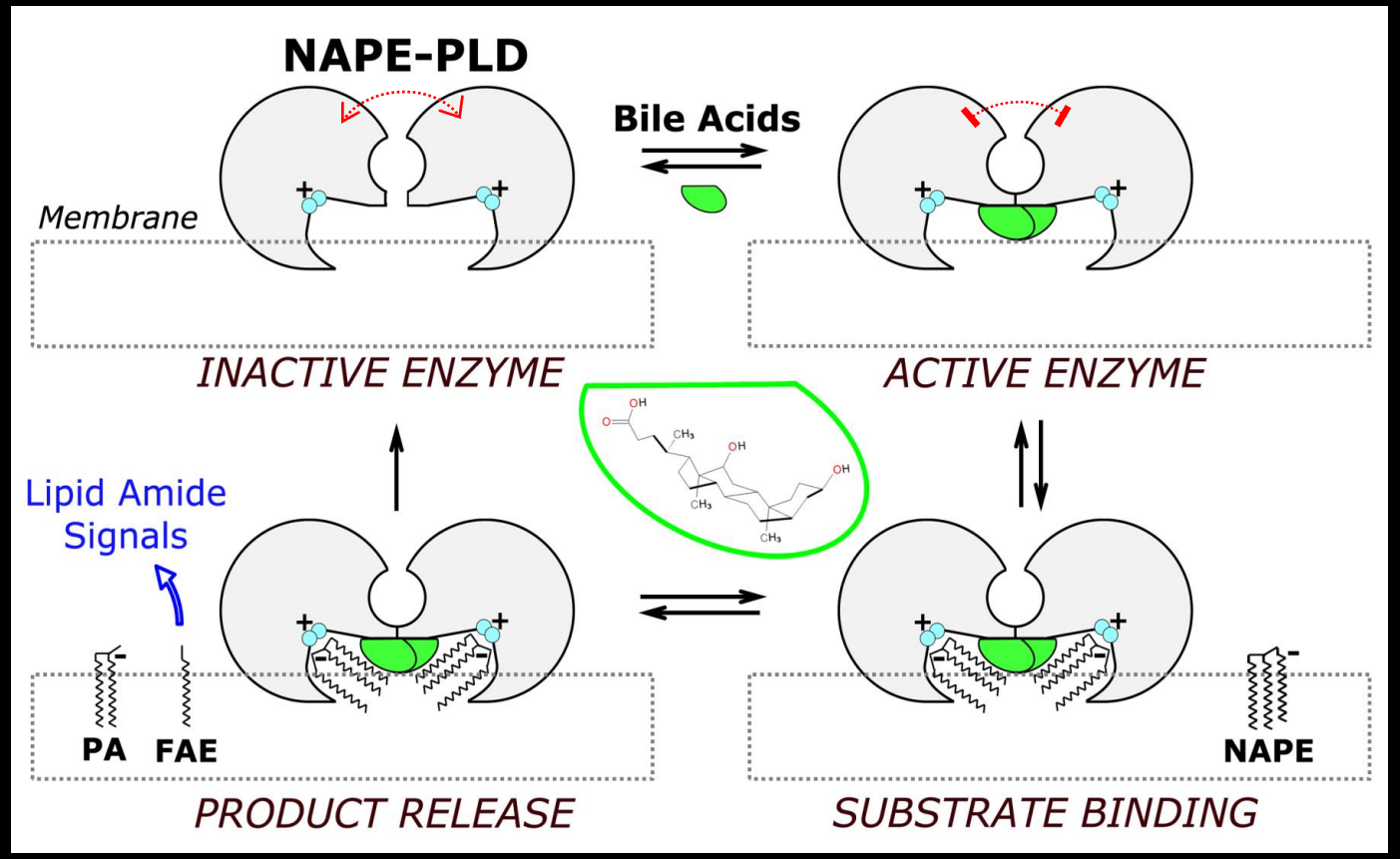






Structure 2015

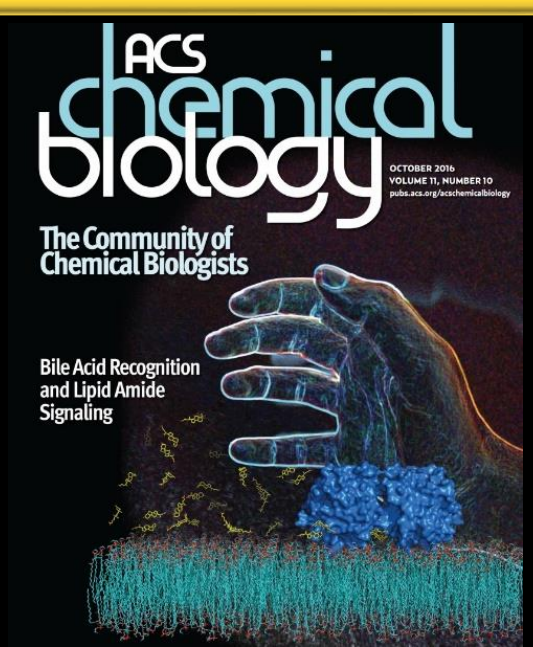
WHAT IS THE ROLE OF BAs?



Dimer stabilization

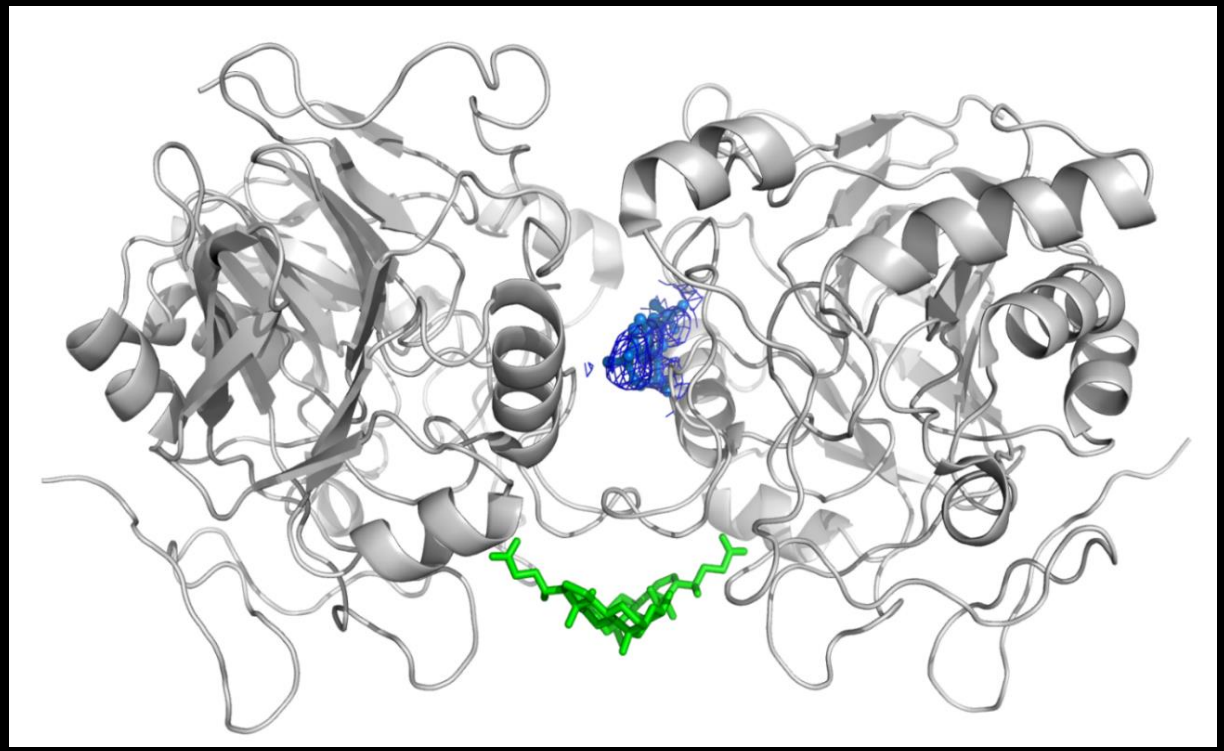
Enzyme activity

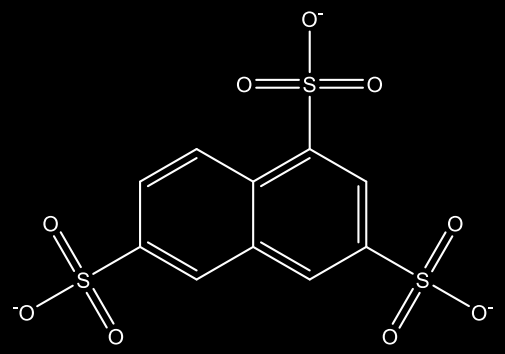
Bile Acids turn NAPE-PLD on



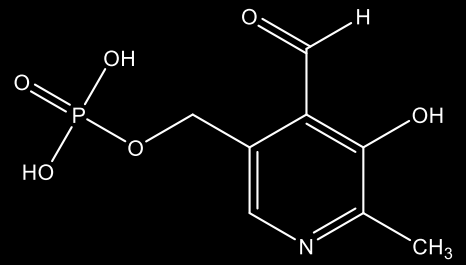
LIGAND DISCOVERY

Ligand screening → SPR → Different Active Classes of Cmps → FRAGMENTS





naphthalene-1,3,6-trisulfonate



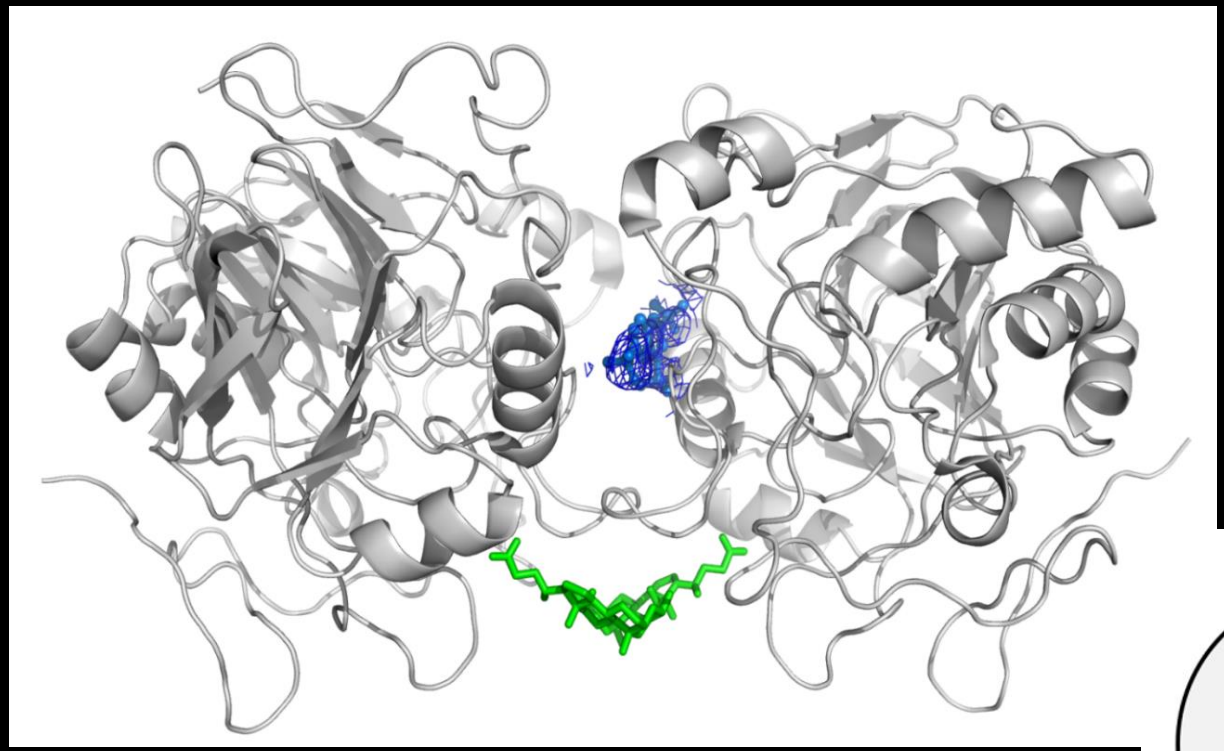
Pyridoxal-5'-phosphate

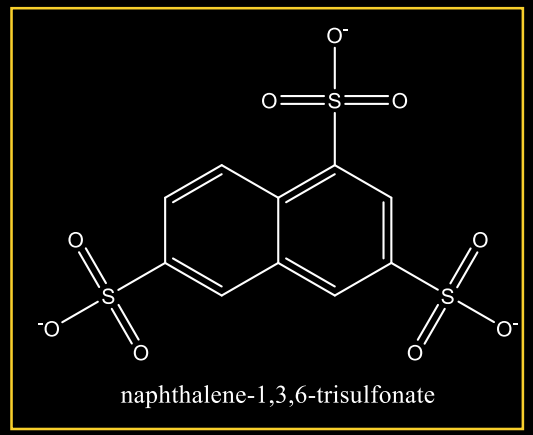
N-(4-hydroxyphenyl)-2,4-dioxo-1,2,3,4,4a,8a-hexahydroquinazoline-6-sulfonamide

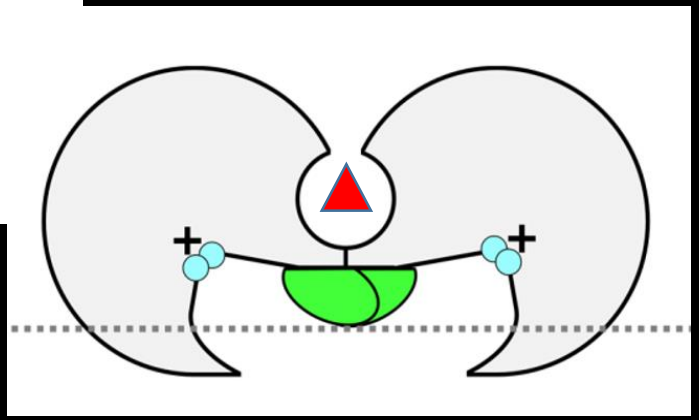
First structures of NAPE-PLD in complex with chemical modulators

Structure of NAPE-PLD in complex with Naph3S

Ligand screening → SPR → Different Active Classes of Cmps → FRAGMENTS







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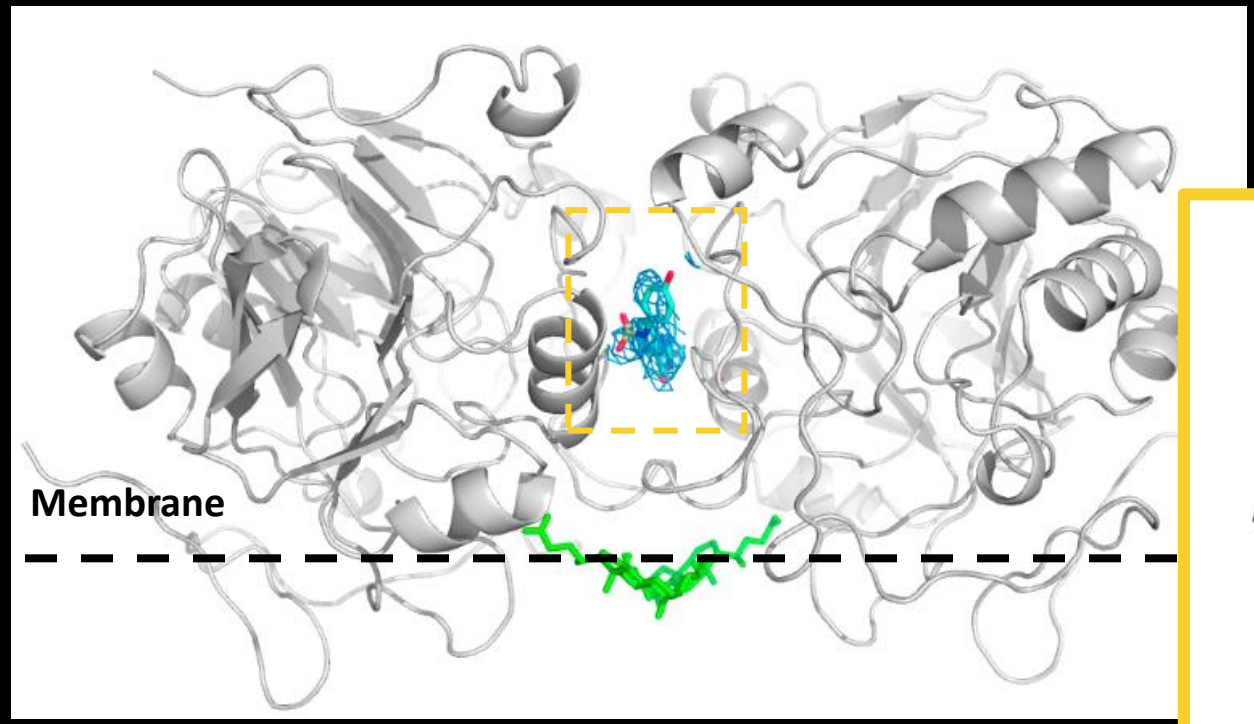
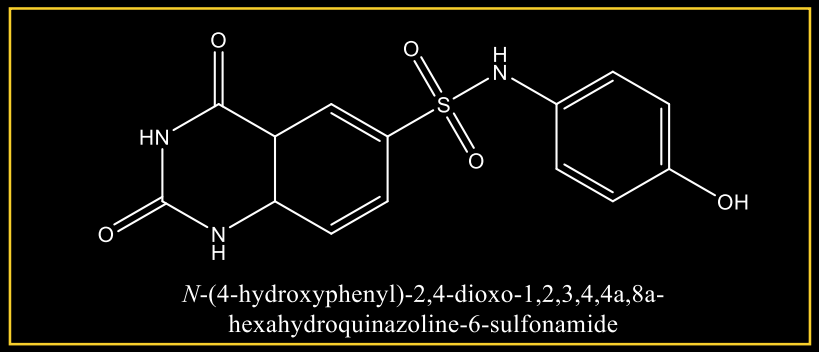
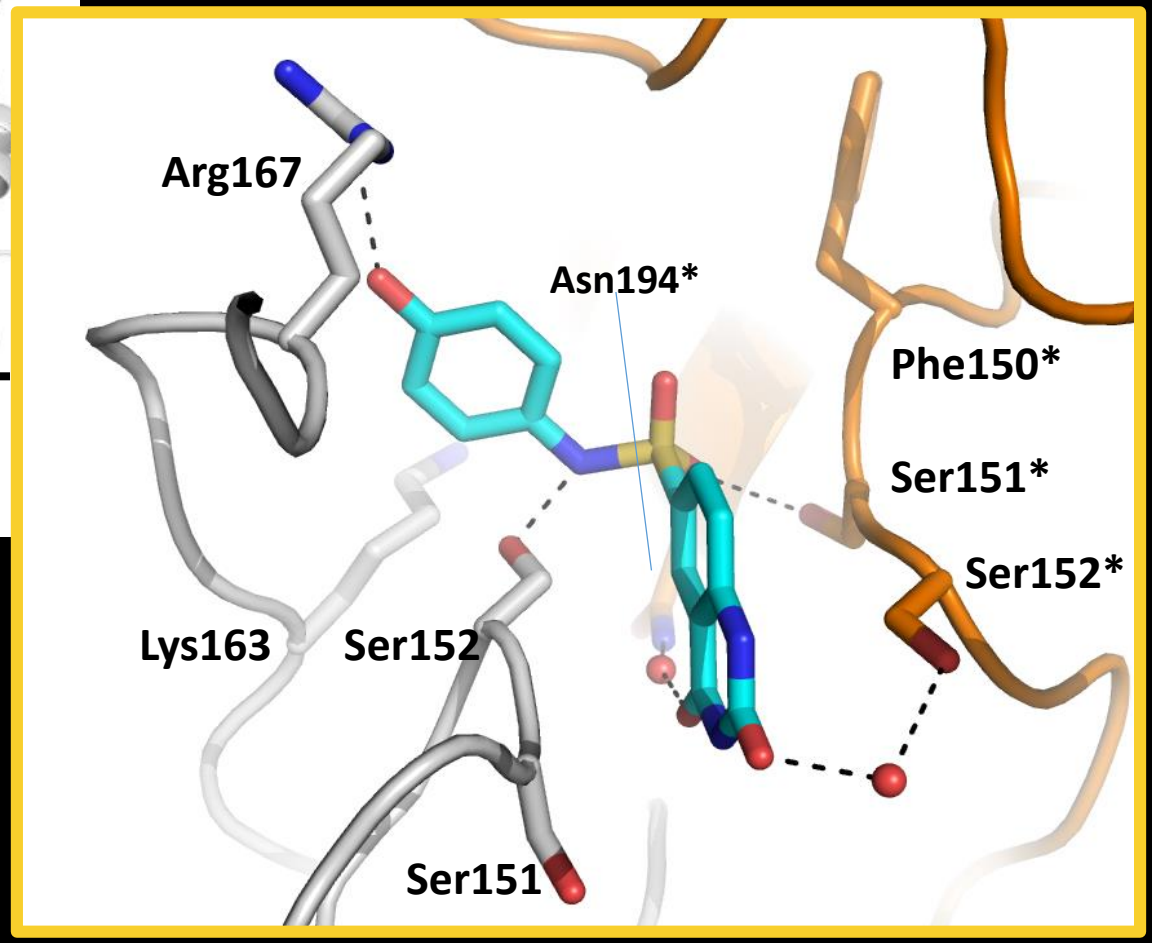
Structure of NAPE-PLD in complex with ARN compound





N-(4-hydroxyphenyl)-2,4-dioxo-1,2,3,4,4a,8a-hexahydroquinazoline-6-sulfonamide



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LIGAND DISCOVERY

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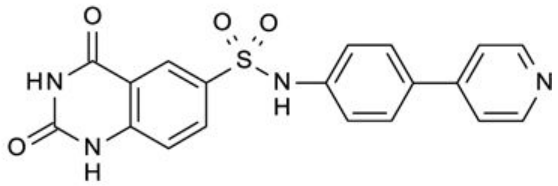
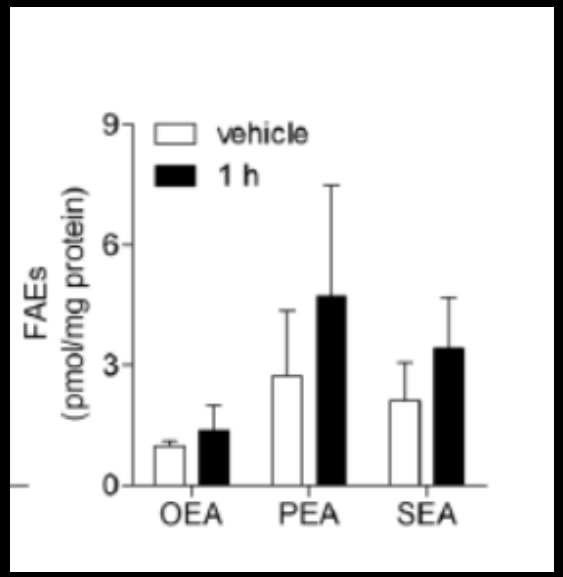
Chem Commun (Camb). 2017 November 28; 53(95): 12814–12817. doi:10.1039/c7cc07582k.

Synthesis and characterization of the first inhibitor of N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD)

Beatrice Castellani[a,±], Eleonora Diamanti[a,#,±], Daniela Pizzirani[a,¥], Piero Tardia[a], Martina Maccesi[c], Natalia Realini[a], Paola Magotti[a,&], Gianpiero Garau[a,†], Thomas Bakkum[a,§], Silvia Rivara[d], Marco Mor[d], and Daniele Piomelli[*,a,b]

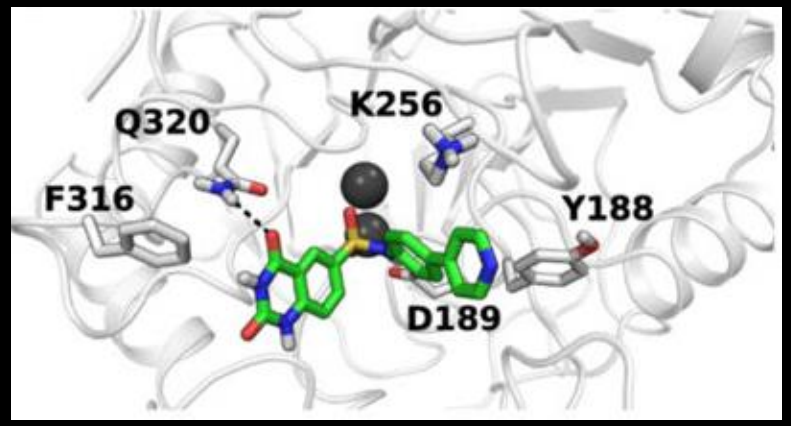


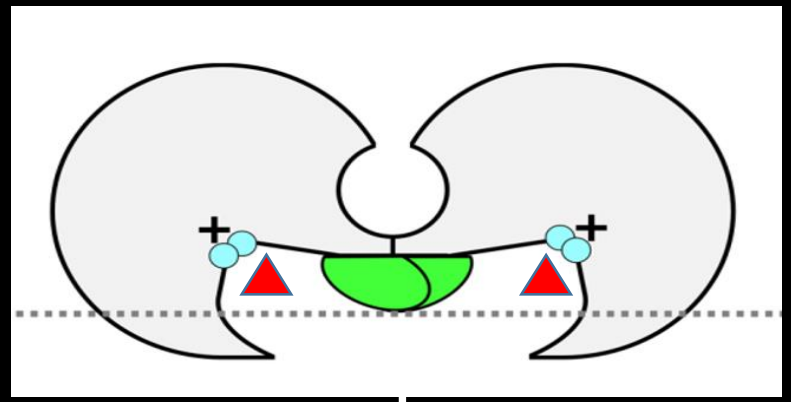
ARN19874
IC50 ≈ 34 μM



NAPE-PLD inhibition (HEK)

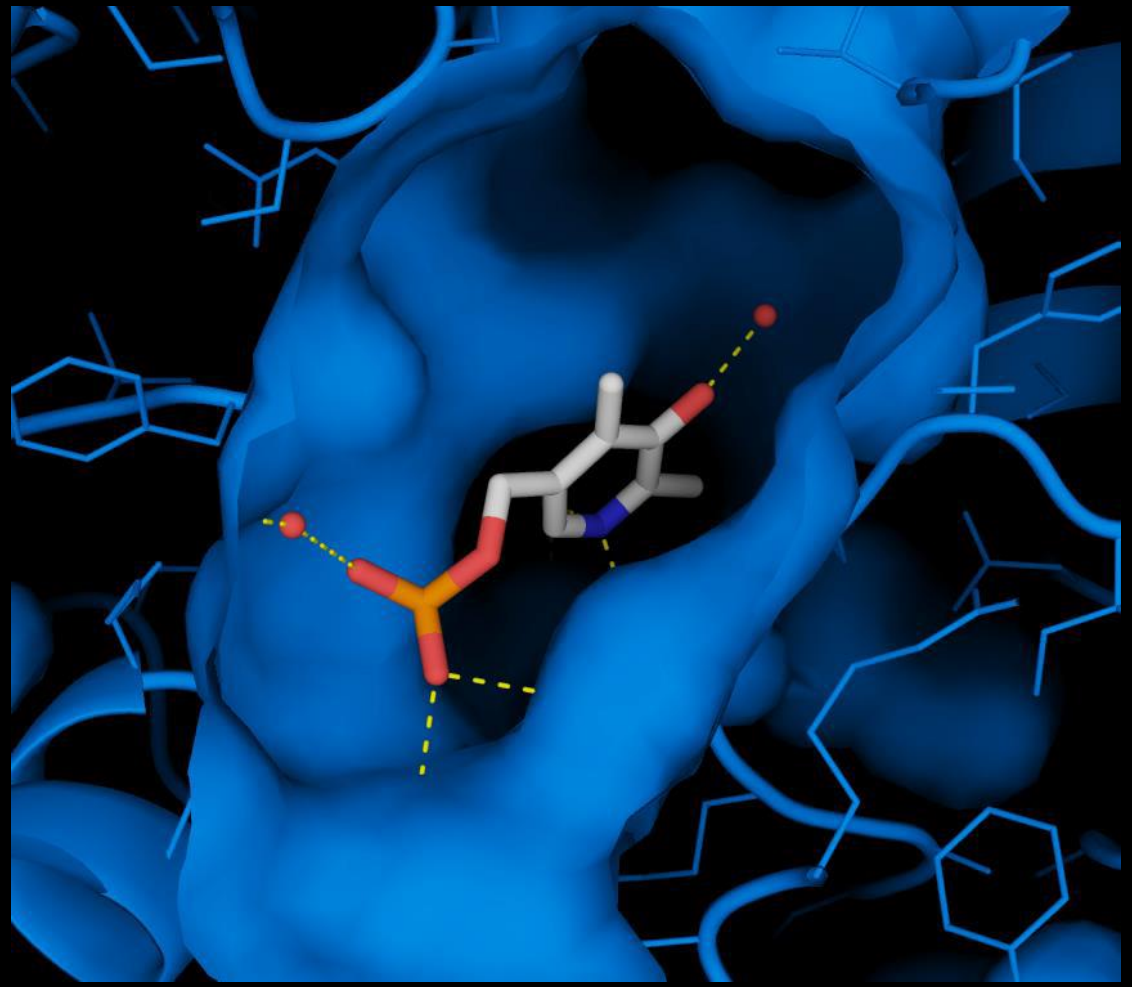
In silico model

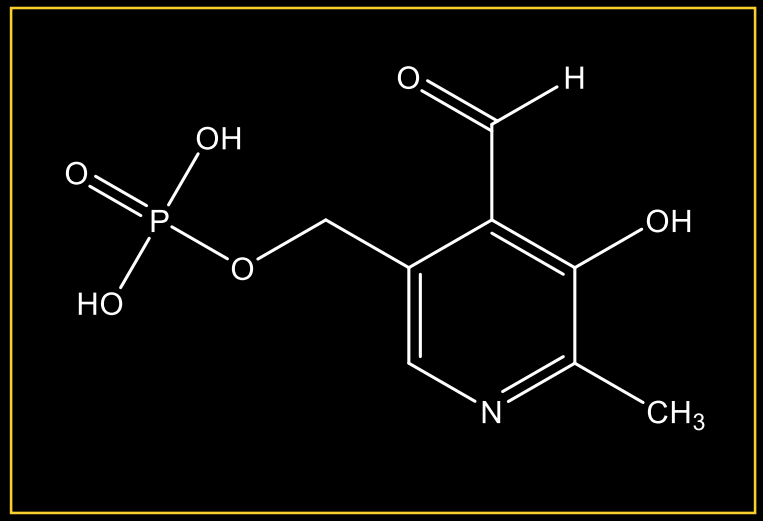




VALIDATION by XRD

Structure of NAPE-PLD in complex with PLP



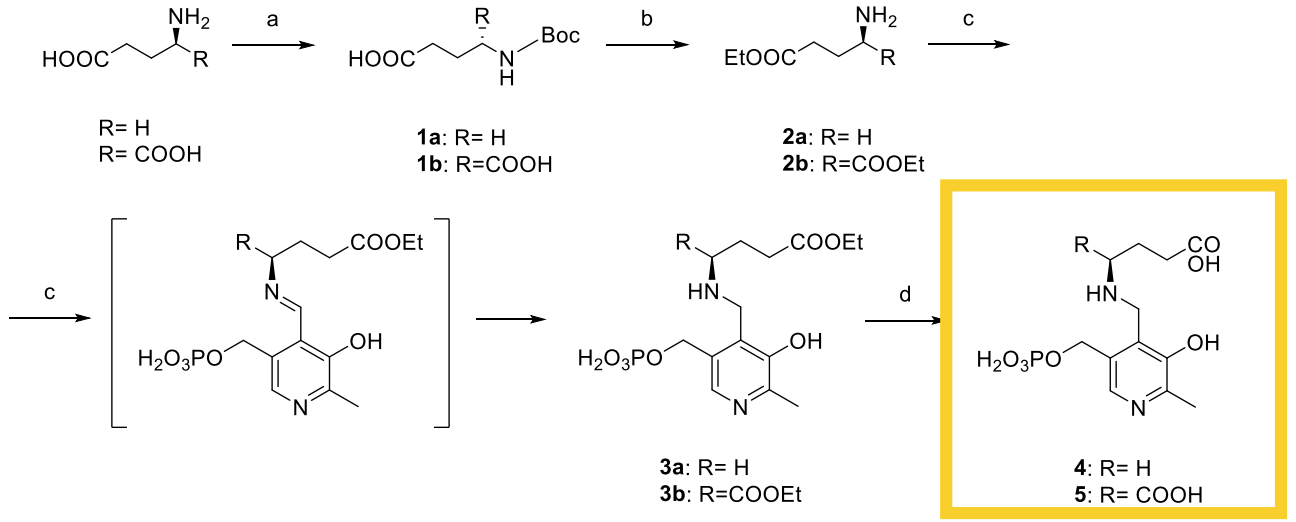


Pyridoxal-5′-phosphate

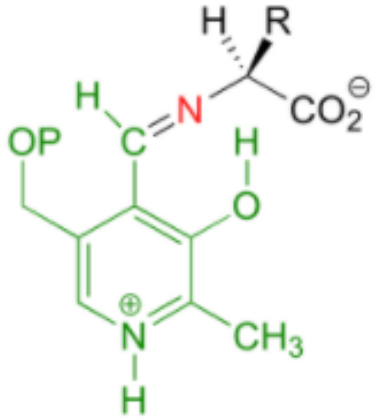
- ☐ Active form of VitaminB6
- ☐ Involved in neurotransmitters generation
- ☐ lipid metabolism and gene expression

PLP ANALOGUES

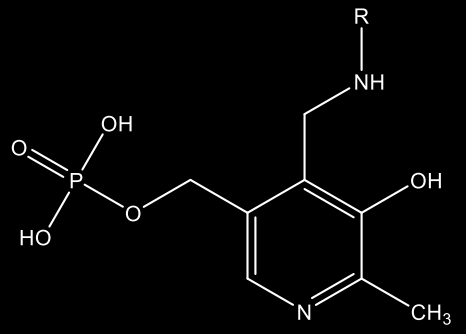
SCHEME 1



Reagents and conditions: a. $N(Et)_3$, Di-tert-butyl dicarbonate, DMF/H_2O, 12 h; **b.** EtOH, $SOCl_2$,r.t., 72 h; **c. 1)** pyridoxal-5′-phosphate, MeOH, 30 min; **2)** $NaBH_4$, 5 °C, 15 min; **3)** 25 °C, 72 h d. EtOH, NaOH 10%, 69 °C , 12 h.

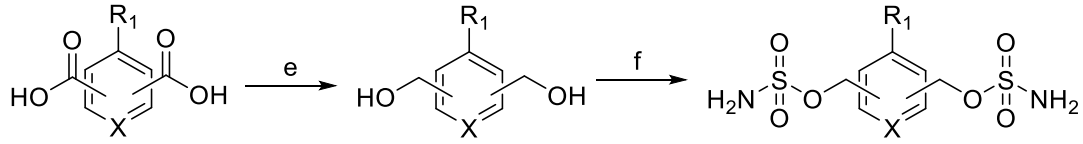


amino acid-PLP



Prof. Simona Rapposelli
University of Pisa
Department of Pharmacy

SCHEME 2



Reagents and conditions: e., $LiAlH_4$, dry THF, 20 h, 0 °C; **f.** sulfamoyl chloride, DMA, r.t.

CONCLUSIONS & PERSPECTIVES

Affinity/kinetic of PLP-derivative

Dr. Aldo Moscardini

Characterization of the structural complex by XRD

Modulation in cell and in vivo

Universiteit Leiden

Prof. Mario van der Stelt

GeCrySchool

HOME @EVENT

"Insights into human membrane NAPE-PLD interactions" (manuscript in preparation)

Universiteit Leiden

Prof. Mario van der Stelt

nature chemical biology

ARTICLES

https://doi.org/10.1038/s41589-020-0528-7

Check for updates

Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice

Elliot D. Mock[1], Mohammed Mustafa[2], Ozge Gunduz-Cinar[3], Resat Cinar[4], Gavin N. Petrie[5], Vasudev Kantae[1,6], Xinyu Di[6], Daisuke Ogasawara[7], Zoltan V. Varga[8], Janos Paloczi[8], Cristina Miliano[9], Giulia Donvito[2], Annelot C. M. van Esbroeck[1], Anouk M. F. van der Gracht[1], Ioli Kotsogianni[1], Joshua K. Park[4], Andrea Martella[1], Tom van der Wel[1,10], Marjolein Soethoudt[1], Ming Jiang[1,10], Tiemen J. Wendel[1], Antonius P. A. Janssen[1,10], Alexander T. Bakker[1], Colleen M. Donovan[3], Laura I. Castillo[3], Bogdan I. Florea[11], Jesse Wat[12], Helma van den Hurk[12], Matthias Wittwer[13], Uwe Grether[13], Andrew Holmes[3], Constant A. A. van Boeckel[1,12], Thomas Hankemeier[6], Benjamin F. Cravatt[7], Matthew W. Buczynski[9], Matthew N. Hill[5], Pal Pacher[8], Aron H. Lichtman[2,14] and Mario van der Stelt[1,10] ✉