Biology of PitVipers-4 Conference
13-16 July 2022 | Geronimo Event Center, Chiricahua Desert Museum
Rodeo, New Mexico USA

Synthesis and *In-Silico* Modeling of Novel Bis-phenyltetrazole Drugs for Inactivation of Rattlesnake Venom Components

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

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Outline…

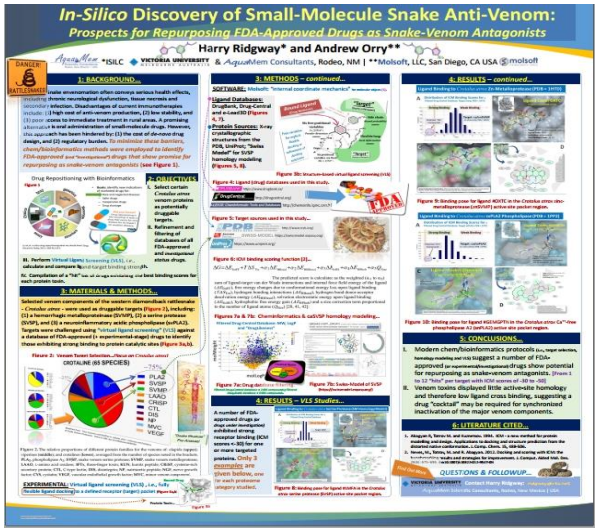
1. Background Work
2. Venom Components (drug targets)
3. Drug Screening
   (Virtual Ligand Screening)
4. Observations (results)
5. Conclusions

Goals & Background…

Goal: Identify novel small-molecule drugs that can efficiently inactivate biotoxins with minimum side effects.

Biology of PitVipers-3 Conference

11 July – 14 July 2019



Quick Summary

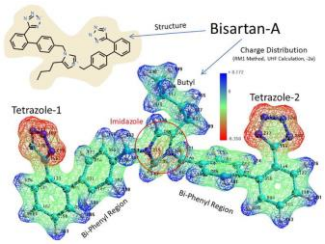
- ✓ Screened 6656 drugs (DrugBank)
- ✓ Targets: svMP, svPLA2, serMP
- ✓ **Identified ~10 promising drugs**
- ✓ No catalytic pocket homology
- ✓ Thus, drug cocktail likely needed

Background: recent research into novel anti-hypertensive drugs…

› Comput Struct Biotechnol J. 2022;20:2091-2111. doi: 10.1016/j.csbj.2022.04.010. Epub 2022 Apr 9.

Discovery of a new generation of angiotensin receptor blocking drugs: Receptor mechanisms and in silico binding to enzymes relevant to SARS-CoV-2

Harry Ridgway [1 2], Graham J Moore [3 4], Thomas Mavromoustakos [5], Sotirios Tsiodras [6], Irene Ligielli [5], Konstantinos Kelaidonis [7], Christos T Chasapis [8 9], Laura Kate Gadanec [10], Anthony Zulli [10], Vasso Apostolopoulos [10 11], Russell Petty [12], Ioannis Karakasiliotis [13], Vassilis G Gorgoulis [12 14 15 16 17], John M Matsoukas [4 7 10]

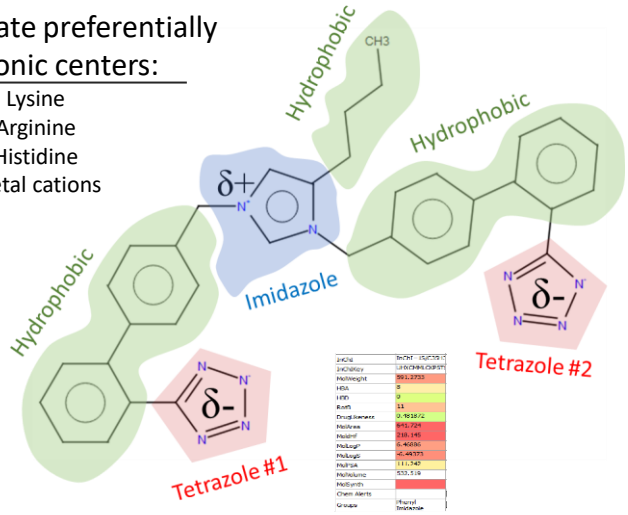


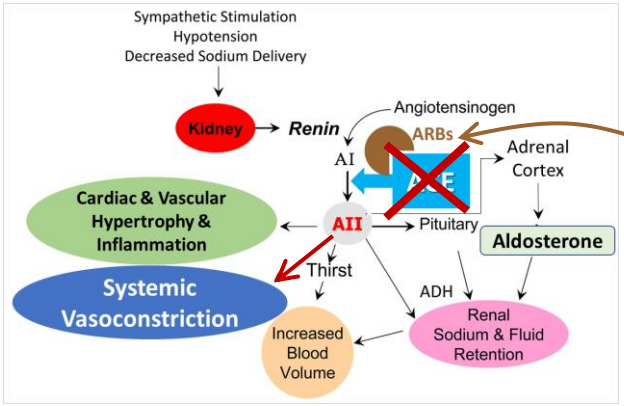
→lab testing shows strong antiviral activity against SARs-CoV-2

Example…Imidazole-Biphenyltetrazole…"Bisartan-A"

ARBs coordinate preferentially with cationic centers:

- ✓ Lysine
- ✓ Arginine
- ✓ Histidine
- ✓ Metal cations



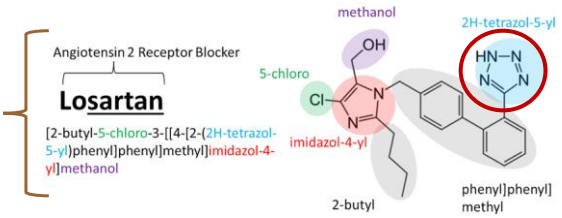


Renin-Angiotensin-Aldosterone-System (RAAS):
[regulation of blood pressure]

Angiotensin-II Receptor Blockers (ARBs) = Drugs that lower blood pressure

"Sartans"

EXAMPLE: ARBs lower blood pressure by stopping formation of Ang-II, a hormone that increases blood pressure.

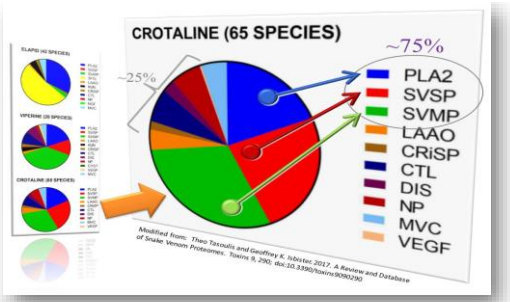


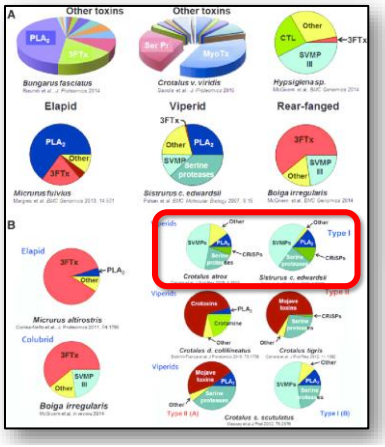
Venom Components…

"Drug Targets"

Venom Components…
"Drug Targets"

Understanding Biological Roles of Venoms Among the Caenophidia: The Importance of Rear-Fanged Snakes: Integrative and Comparative Biology 56(5) | DOI:10.1093/icb/icw110 | Authors: Stephen P Mackessy, University of Northern Colorado | Anthony Saviola, University of Colorado





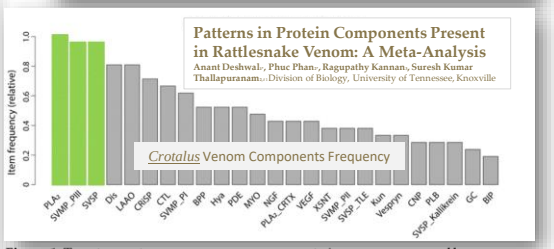
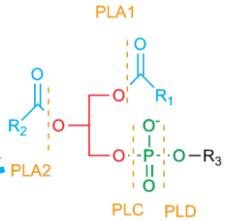


Figure 1. Twenty most common venom components in venom expressed by genus: *Crotalus*. PLA2 and SVSP are identified as the most common amongst *Crotalus* species (relative frequency is 1). *Note*: 5′-nucleotidase (5′-NT), Bradykinin inhibitory peptide (BIP), Bradykinin potentiate peptide (BPP), Ctype Lectins (CTL), Natriuretic peptide type C (CNP), Crotoxin (CRTX), Disintegrin (Dis), Guanylyl cyclase (GC), Hyaluronidase (Hya), Kunitz-type inhibitor (Kun), L-amino acid oxidase (LAAO), Myotoxin (MYO), Nerve growth factor (NGF), Phosphodiesterase (PDE), Phospholipase A2 (PLA2), Phospholipase B (PLB), Snake venom metalloprotease (SVMP), Snake venom serine protease (SVSP), Vascular endothelial growth factor (VEGF).

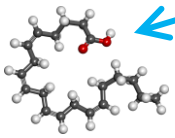
Phospholipase A2 (PLA2)

Phospholipases A2 (PLA2s) EC 3.1.1.4: Cleave fatty acids at sn-2 position of glycerophospholipids, hydrolyzing the bond between the second fatty acid "tail" and glycerol…



lysophosphatidic acid

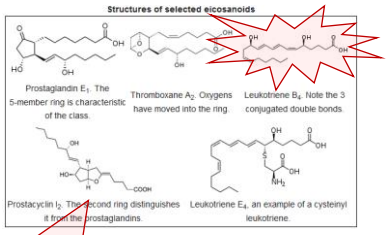
Arachidonic Acid



Eicosanoids: Signaling molecules made by the enzymatic or non-enzymatic oxidation of arachidonic acid or other polyunsaturated fatty acids (PUFAs) that are, similar to arachidonic acid, 20 carbon units in length.

Enzymatic oxidation
(new targets???)

Eicosanoids
signaling

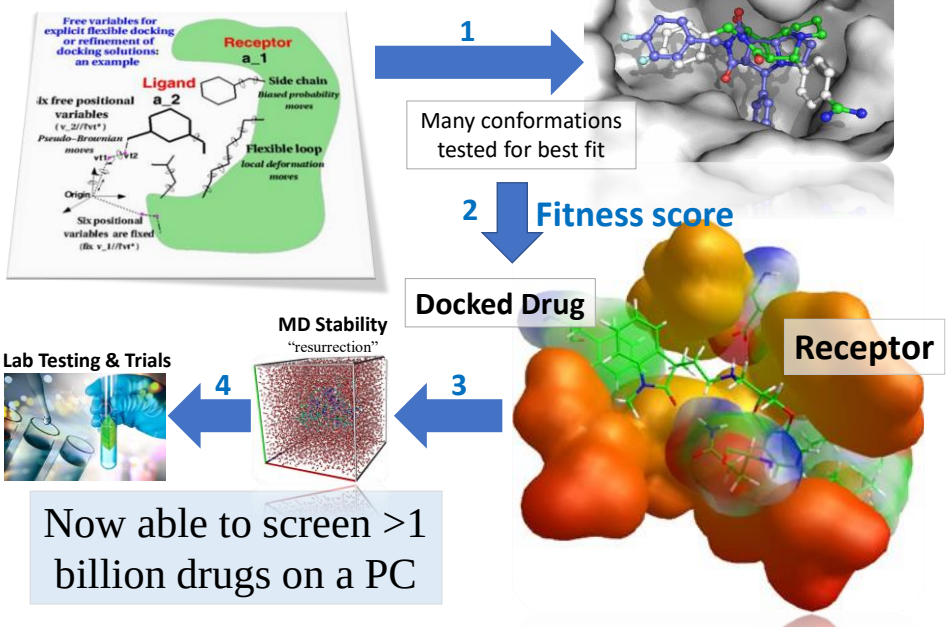


Prostaglandins
anti-inflammatory

Leukotrienes
pro-inflammatory

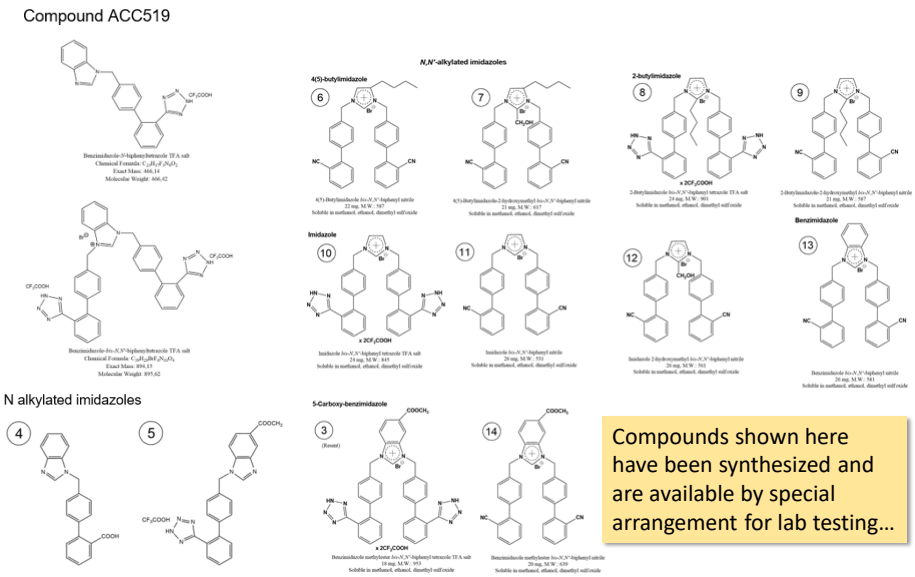
A word about methods…

Drug Docking and VLS…



Focused Chemical (Drug) Space → *FDA-approved & novel ARBs*

+PLA2 known inhibitor ***Varespladib*** **(36 total ligands)**



Docking to svPLA2…

Crotalus atrox PLA2 [with inserted Ca^{2+} ions]

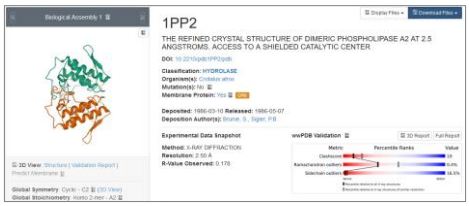
The refined crystal structure of dimeric phospholipase A2 at 2.5 A. Access to a shielded catalytic center…

Brunie, S., Bolin, J., Gewirth, D., Sigler, P.B.

(1985) J Biol Chem 260: 9742-9749

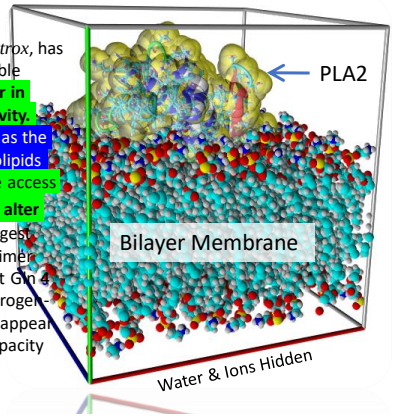
PubMed: 4019493 Search on PubMed

Primary Citation of Related Structures: **PDB 1PP2**



PubMed Abstract: *Crotalus atrox*

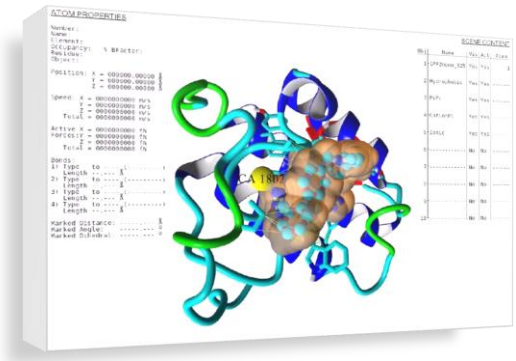
The 2.5-A crystal structure of the calcium-free form of the dimeric venom phospholipase A2 from the Western Diamondback rattlesnake *Crotalus atrox*, has been refined to an R-factor of 17.8% (I greater than 2 sigma) and acceptable stereochemistry. **The molecule is a nearly perfect 2-fold symmetric dimer in which most of the catalytic residues of both subunits face an internal cavity. The restricted access to the putative catalytic sites is especially puzzling** as the optimal substrates for this and most other phospholipase A2 are phospholipids condensed in micellar or lamellar aggregates. We point out that substrate access to **the internal cavity may be aided by calcium binding which can alter the inter-subunit contacts that shield the catalytic network.** We also suggest that a system of hydrogen-bonded moieties exists on the surface of the dimer that links the amino terminus to the catalytic system, through an invariant Gln 4 side chain and the backbone of the active center residue, Tyr 73. This hydrogen-bonded network is on a highly accessible surface of the dimer and would appear to contribute to the enzyme's (as opposed to the proenzyme's) special capacity to attack aggregated rather than monomeric substrate.



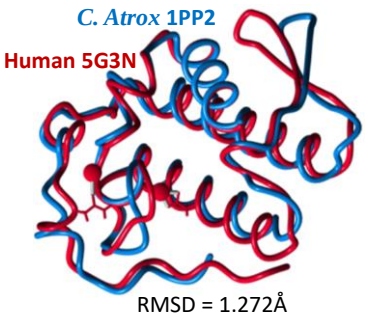
A-Chain of Dimeric svPLA2 (PDB 1PP2), *Crotalus atrox*

+ 2 x Ca^{2+} ions from superposition of human PLA2 (PDB 5G3N)

Calcium co-factor isoform of C. atrox PLA2…



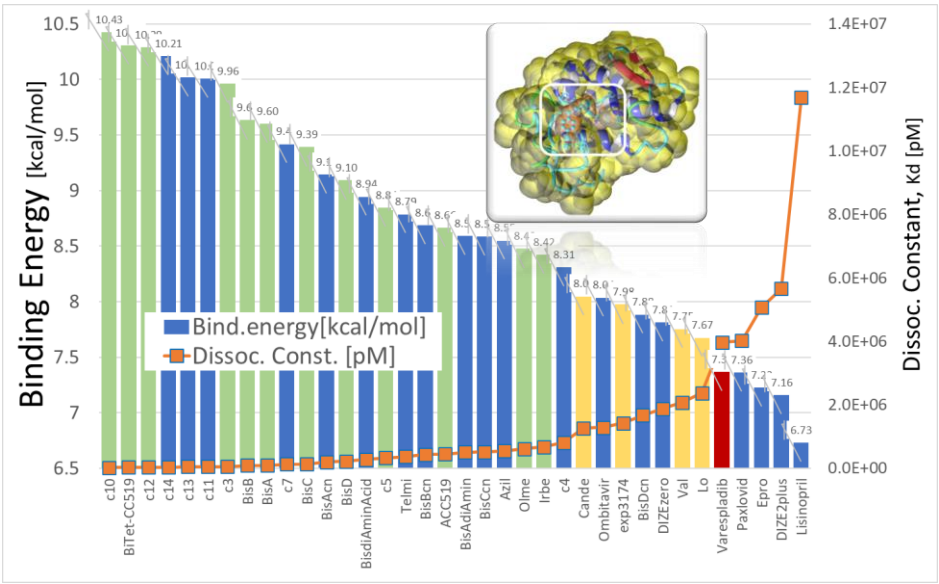
https://www.rcsb.org/structure/1PP2



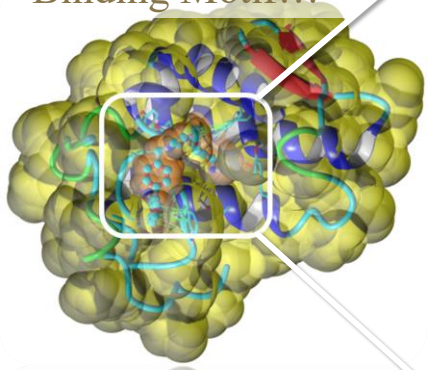
RMSD = 1.272Å

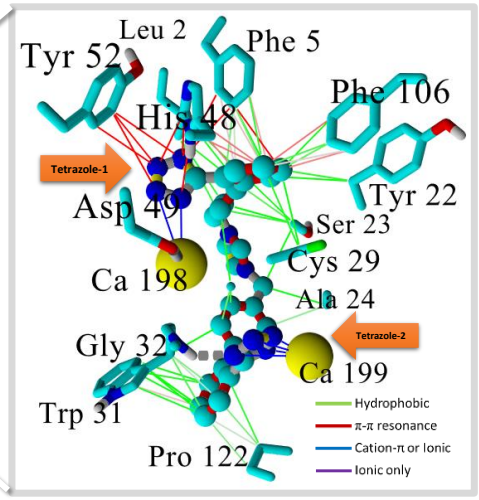
Ligand docking to *C. atrox* 1PP2:

2 x Ca^{2+} ions inserted from human PLA2 (PDB 5G3N)…

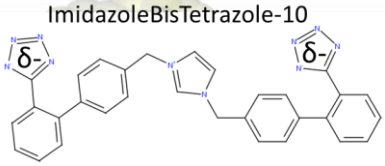


Compound "C10" Binding Motif…





ImidazoleBisTetrazole-10



Dominant Interactions:

- ✓ **Hydrophobic**
- ✓ **pi-pi resonances**
- ✓ **Cation-pi** (with Ca2+ ions)

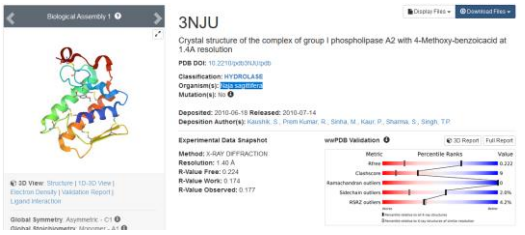
test

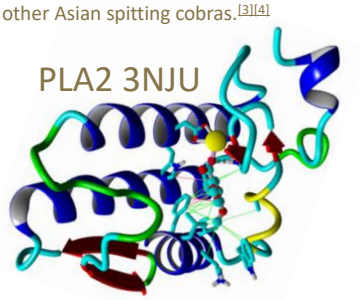
We “nailed” the LD50…!

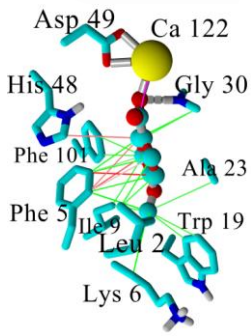
Manuscript in preparation…!

Docking to Ca^{2+} -Dependent svPLA2…*Naja sagittifera*

The **Andaman cobra** or **Andaman spitting cobra** (*Naja sagittifera*) is a species of cobra endemic to the Andaman Islands of India. The name of this cobra comes from the Islands itself. It is venomous and also has the ability to spit its venom, although not as fairly well as other Asian spitting cobras.[3][4]







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2016; 4(1): 10-14
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doi: 10.11648/j.cbb.20160401.12
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SciencePG
Science Publishing Group

The Role of Calcium on the Active Site of Snake Venom Phospholipase A2: Molecular Dynamics Simulations

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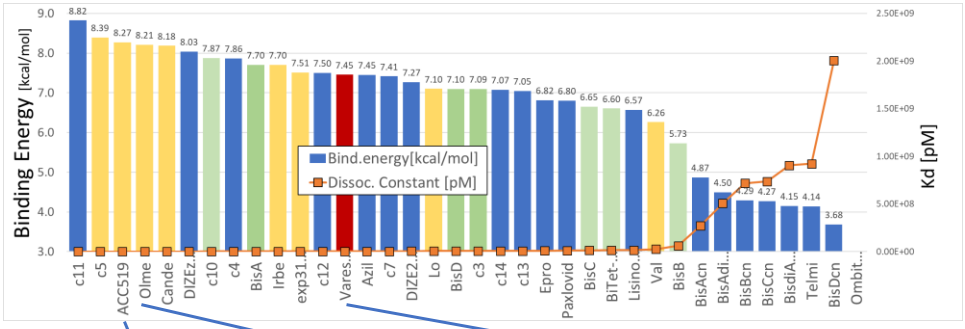
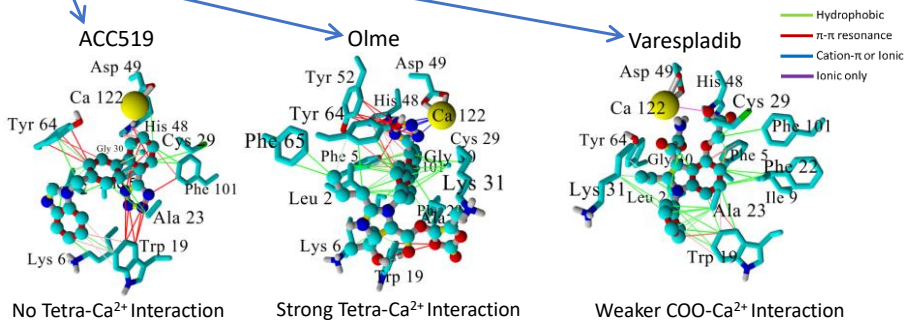
[2]Department of Biochemistry, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria
[3]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract: Snake venoms are rich in phospholipase A2 (PLA2) and their hydrolysis of cell membrane phospholipids explains the role of the enzyme in venom toxicity. Calcium is known to plays important role at the active site of PLA2 during catalysis. In this study, molecular dynamics simulations of free PLA2 and calcium bound PLA2 were carried out using GROMACS 4.5.5 to evaluate the role of calcium in PLA2 catalysis. The results showed that calcium induced formation of helical structures between Arg62 - Lys66, Asn107 - Tyr111 and Asp114 - Cys119 in PLA2 which with time disappeared through the formation and opening of loops. **Calcium induced atomistic movements and conformational changes in snake venom PLA2 which led to the formation of a widened cleft at the active site of calcium bound PLA2 when compared with free PLA2. This could lead to a better binding and accommodation of substrate, thus enhancing catalysis.** This study confirms the role of calcium towards the action of PLA2 in snake venom toxicity and could provide useful information for the design of small molecules that can function as PLA2 inhibitors.

Docking to 3NJU Cobra svPLA2: Some Observed Interactions with Ca^{2+} Co-Factor

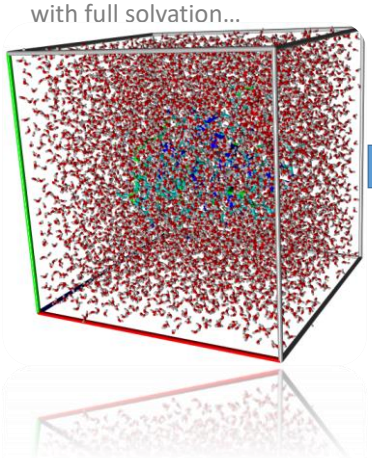




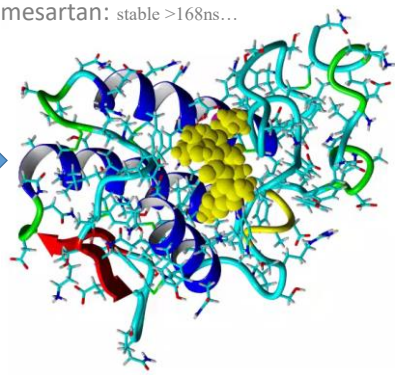
Test Receptor-Drug Complex Stability

molecular dynamics (MD) simulationTIP3P explicit solvation @ 1g/ml | physiol. saline
temp. = 311°K | Amber14 FF parameters | time = 168 nanoseconds

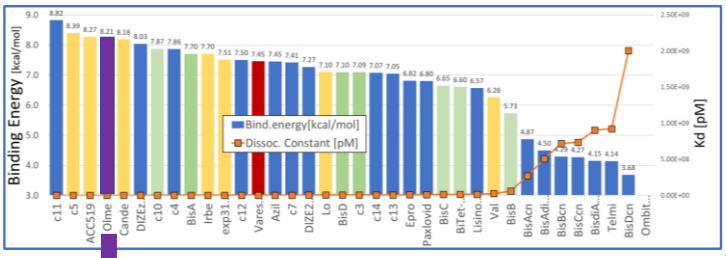
Periodic Boundaries with full solvation…



MD Simulation with Bound Olmesartan: stable >168ns…

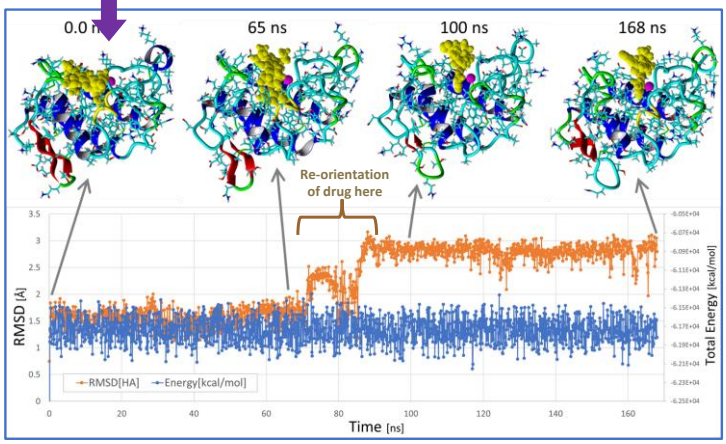


Initial Docking Results...



MD Simulation:
(**Cobra** PLA2/Olmesartan)

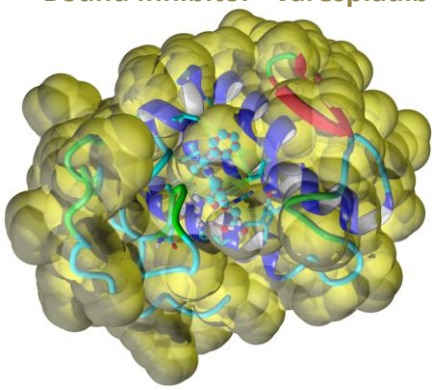
Complex stable at 311°K in physiol. Saline for 168 ns

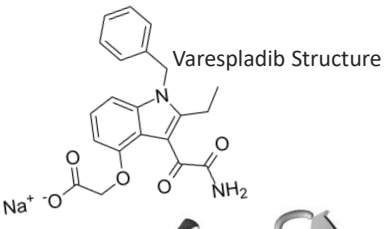


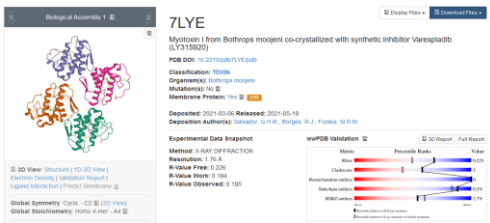
Docking to Ca^{2+}-free Myotoxin-1 from *Bothrops moojeni*...

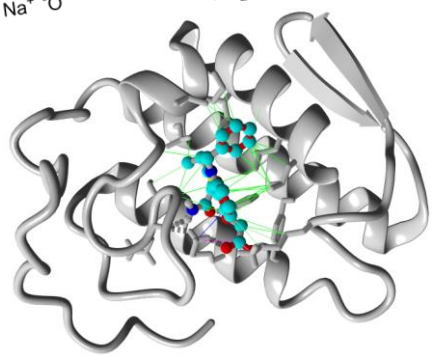
Brazillian Lancehead

Bothrops moojeni Myotoxin-1 7LYE + Bound Inhibitor "Varespladib"…

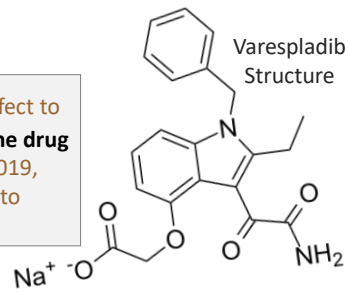








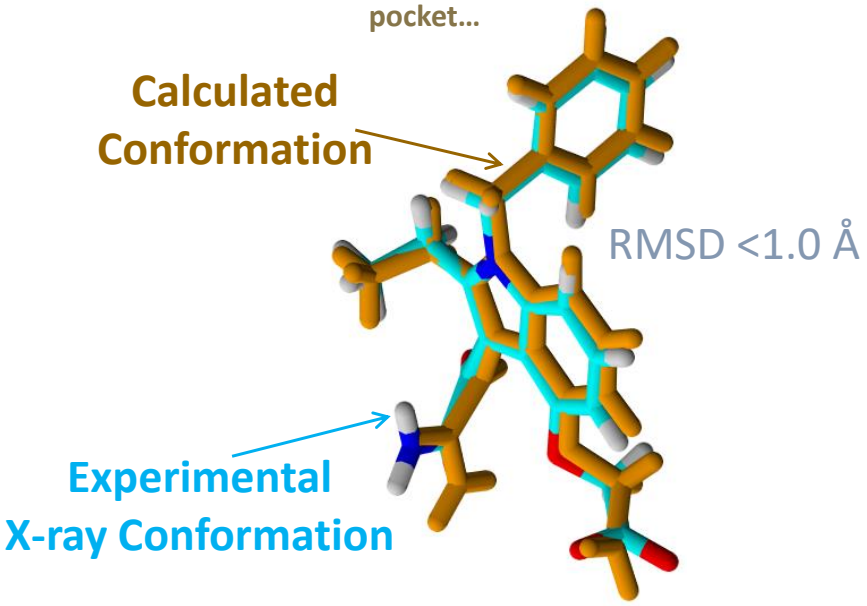
Varespladib showed a significant inhibitory effect to snake venom PLA_2 which makes it a **potential first-line drug candidate in snakebite envenomation therapy.** In 2019, the FDA granted **orphan drug status** for its potential to treat snakebite.

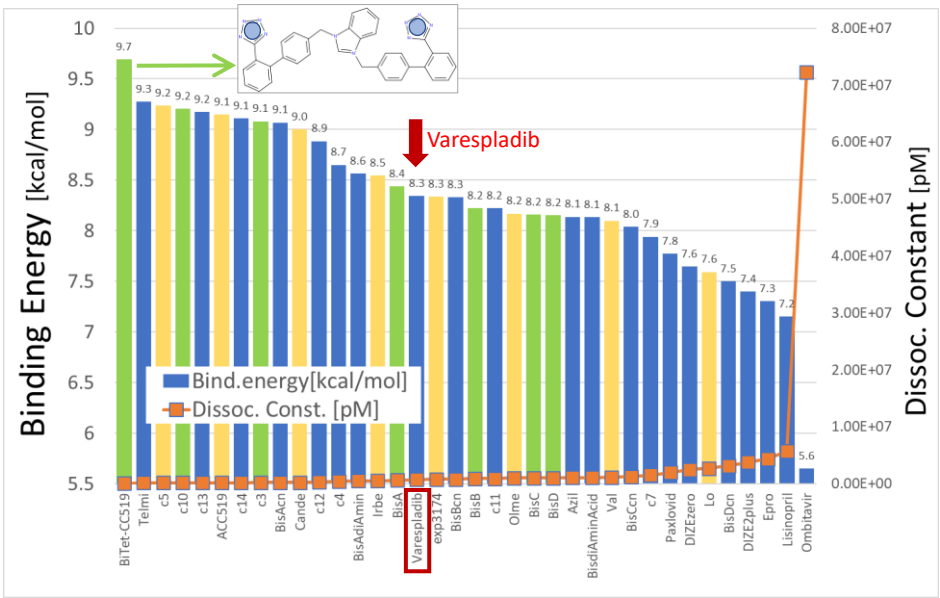


- ✓ Compound belongs to the class of organic compounds known as **phenoxyacetic acid derivatives**. These are compounds containing an anisole where the methane group is linked to an acetic acid or a derivative.

- ✓ **Varespladib is an inhibitor of the IIa, V, and X isoforms of secretory phospholipase A2** (sPLA2).[1][2][3] The molecule acts as an anti-inflammatory agent by disrupting the first step of the arachidonic acid pathway of inflammation.

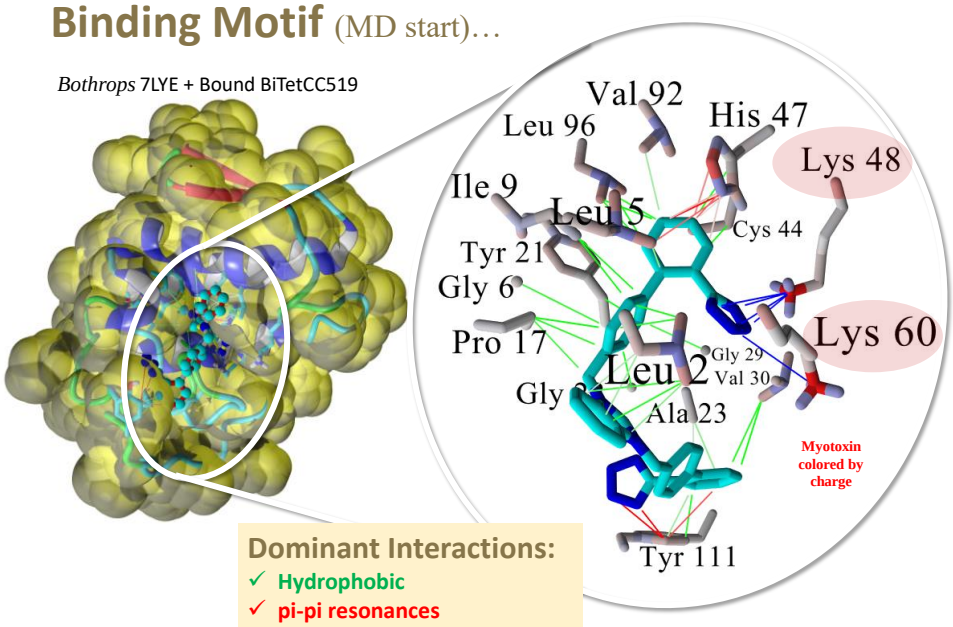
Comparing predicted (VINA) and experimental (x-ray) conformations of bound Varespladib in the 7LYE Myotoxin catalytic pocket…





Binding Motif (MD start)…

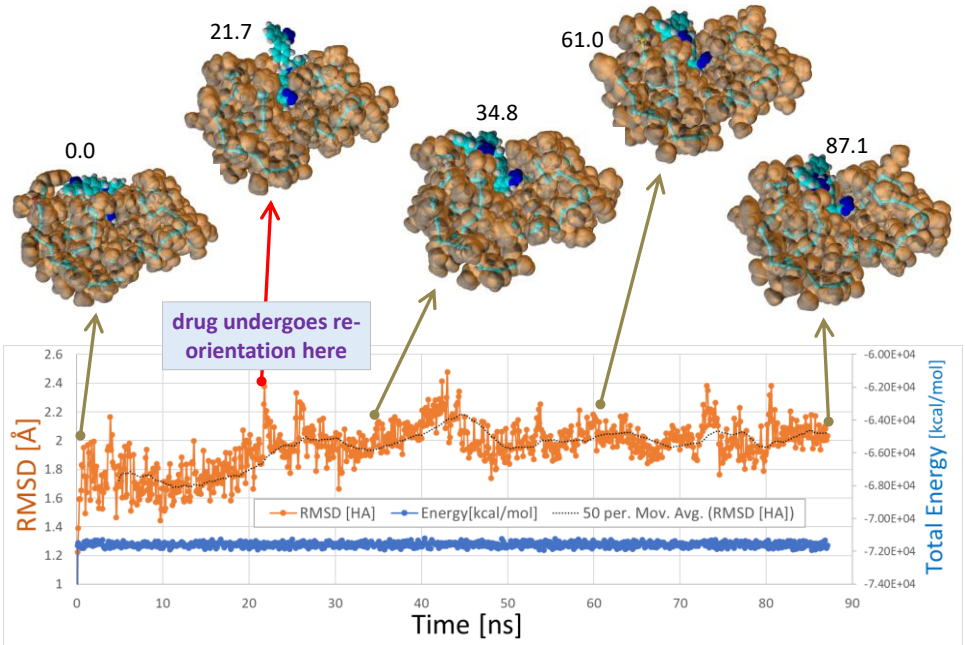
Bothrops 7LYE + Bound BiTetCC519



Dominant Interactions:

- ✓ **Hydrophobic**
- ✓ **pi-pi resonances**
- ✓ **Cation-pi** (with Lys 48 & 60)

Drug was stable but re-oriented…

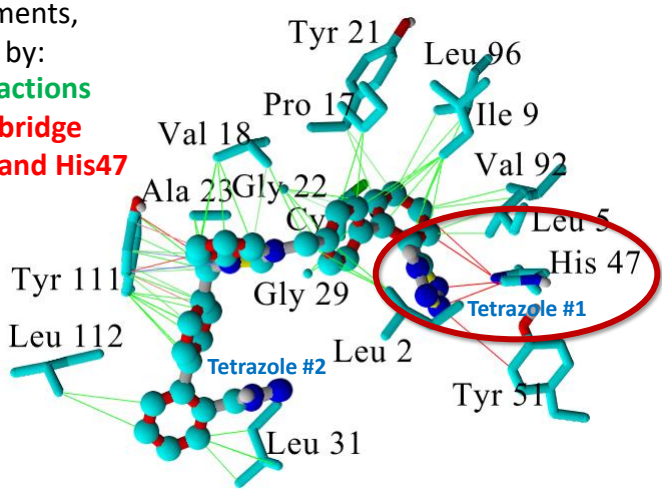


Last Frame of MD Simulation: 87ns

After MD rearrangements, binding mediated by:

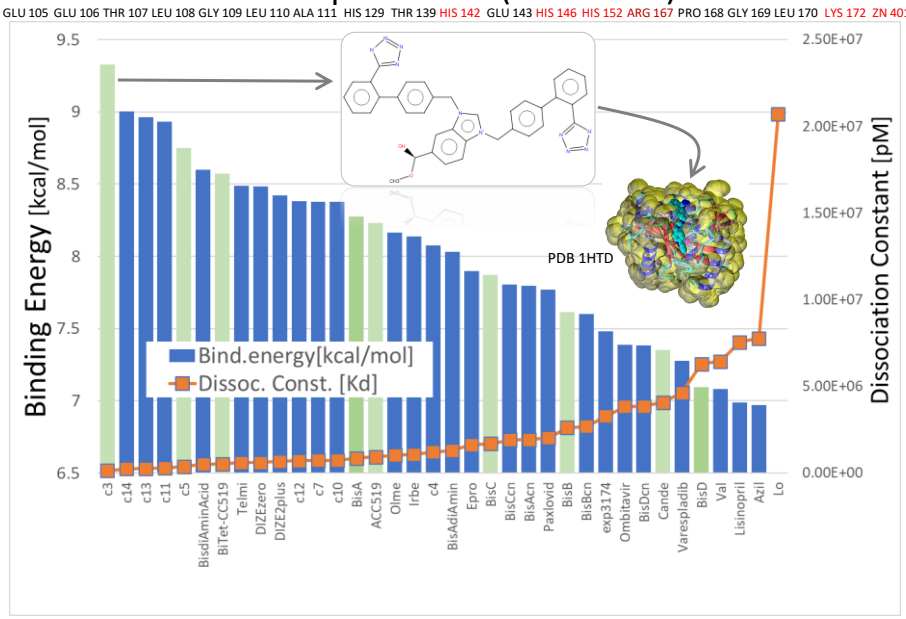
✓ **hydrophobic interactions**

✓ **cation-π and salt bridge between tetrazole-1 and His47**

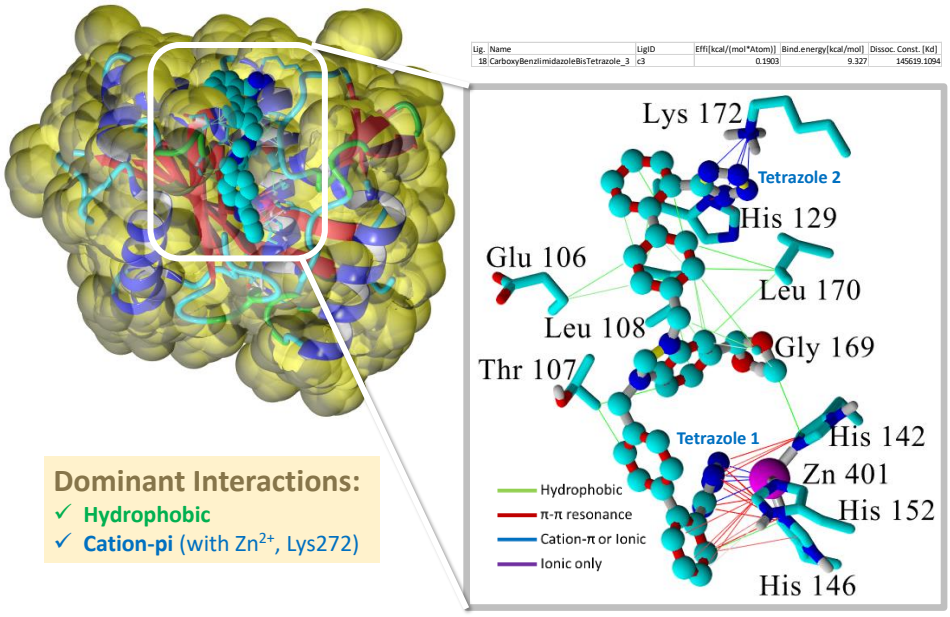


Docking to Zn^{2+} *Crotalus atrox* Metalloproteinase (PDB 1HDT)

Docking of 36 ARBs to Zinc Pocket of *Crotalus atrox* Metalloproteinase (PDB 1HTD)



Docking of Bis-Tetrazole "c3" (yob18) to Zinc Pocket of *Crotalus atrox* Metalloproteinase (PDB 1HTD)



Dominant Interactions:
- ✓ **Hydrophobic**
- ✓ **Cation-pi** (with Zn^{2+}, Lys272)

Quick Summary: Computational Modeling

Target Receptor	Best Ligand	Tetrazole	Binding Energy (kcal/mol) Ligand	Binding Energy (kcal/mol) Varespladib	Stable MD
C. Atrox PLA2 (2xCa2+)	C10	Yes	10.43	7.39	Yes
Cobra PLA2 (1xCa2+)	C11	No	8.82	7.45	Yes
Bothrops Myotoxin (no Ca^{2+})	BiTetCC519	Yes	9.7	8.3	Yes
C. atrox svMP (Zn2+)	C3	Yes	9.35	7.3	not done

Some concluding remarks…

- ✓ **VINA algorithms accurately predict x-ray pose of Varespladib.**
- ✓ **ARBs docked to catalytic sites of variants of svPLA2 and *C. atrox* svMP.**
- ✓ **ARB binding stable over multi-ns MD simulations at 311°K in physiological saline.**
- ✓ **ARB docking stabilized by salt-bridge interactions of anionic tetrazole groups with metal cations and side chains (His, Lys, Arg).**
- ✓ **In absence of metal co-factors (*e.g.*, *Bothrops* Myotoxin), ARB binding stabilized by hydrophobic and pi/pi-resonance.**
- ✓ **Overall, ARBs exhibited robust target binding compared to known svPLA2 inhibitor, Varespladib.**

Thank You!
Questions?