



# Computational approaches in drug discovery

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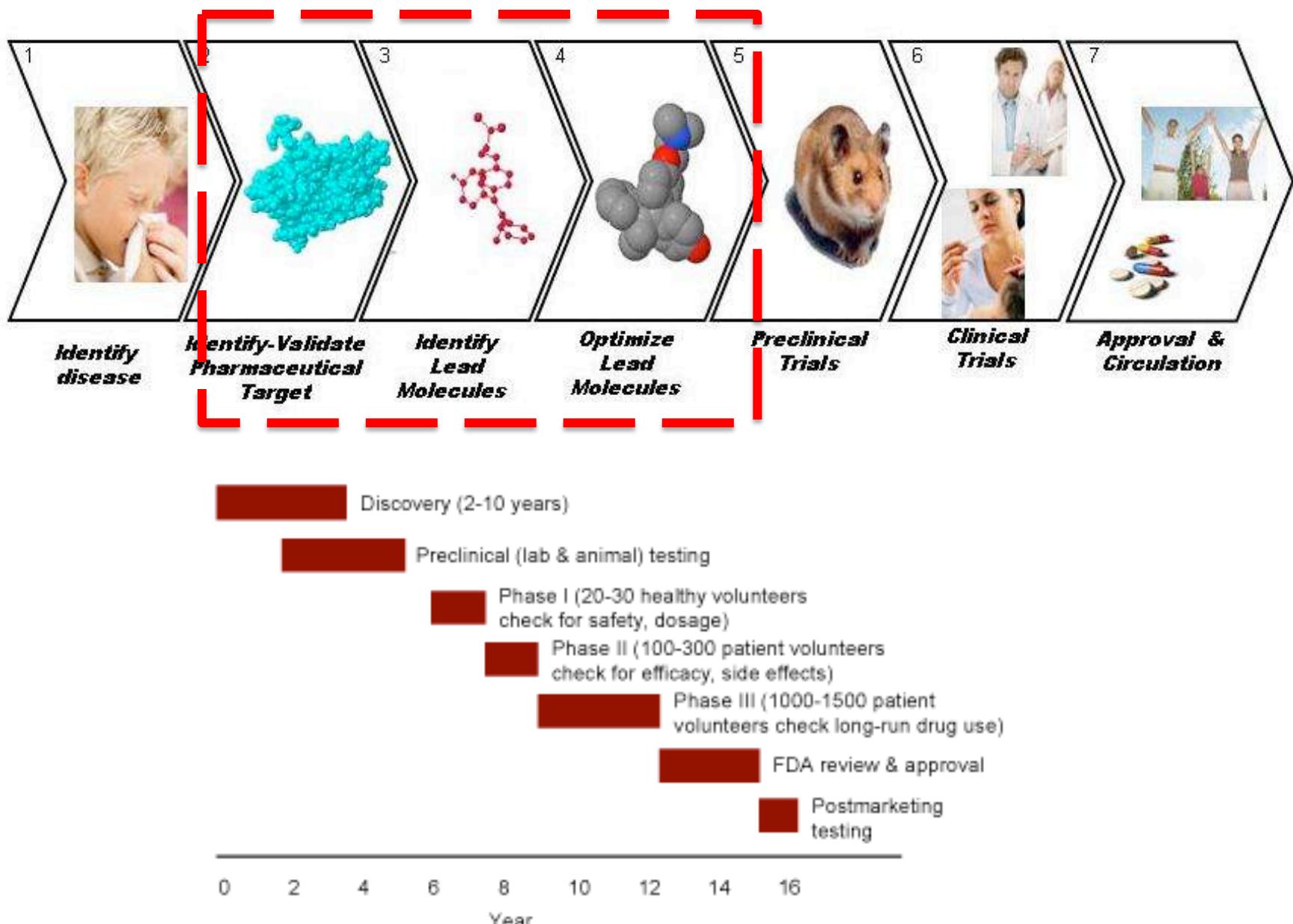
Universidad Católica de Murcia (UCAM), Spain  
<http://www.ucam.edu>



Saturdays.AI, Murcia, 28<sup>th</sup> March 2020

Horacio Pérez Sánchez – [hperez@ucam.edu](mailto:hperez@ucam.edu)

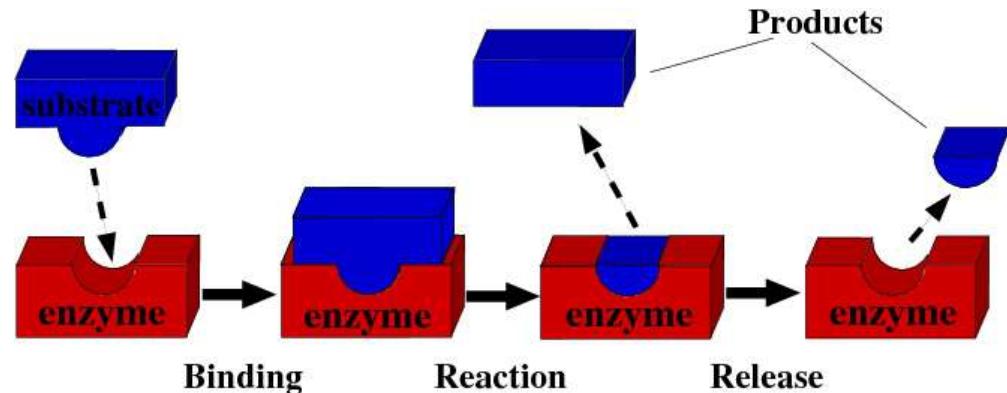
# DRUG DISCOVERY PROCESS



# DISCOVERY OF NEW DRUGS; HOW?

- very specialized for a concrete process
- catalyze chemical reactions

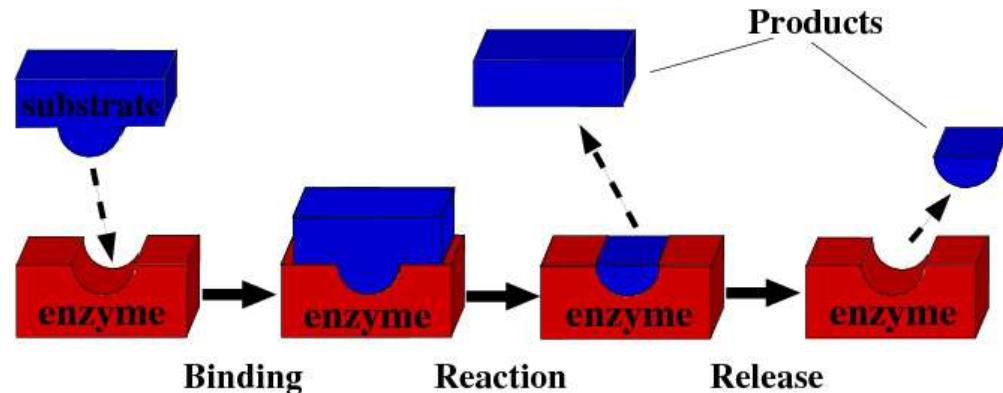
We could be interested  
In blocking this process



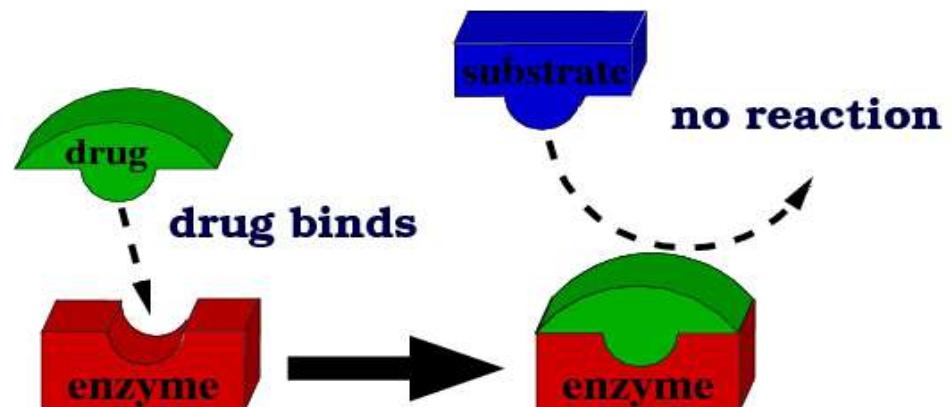
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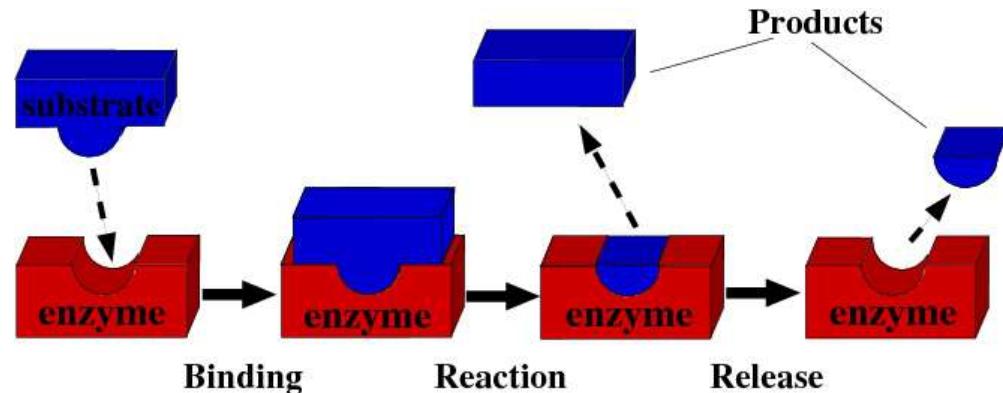
Drug: active molecule with higher affinity than natural ligand



# DISCOVERY OF NEW DRUGS; HOW?

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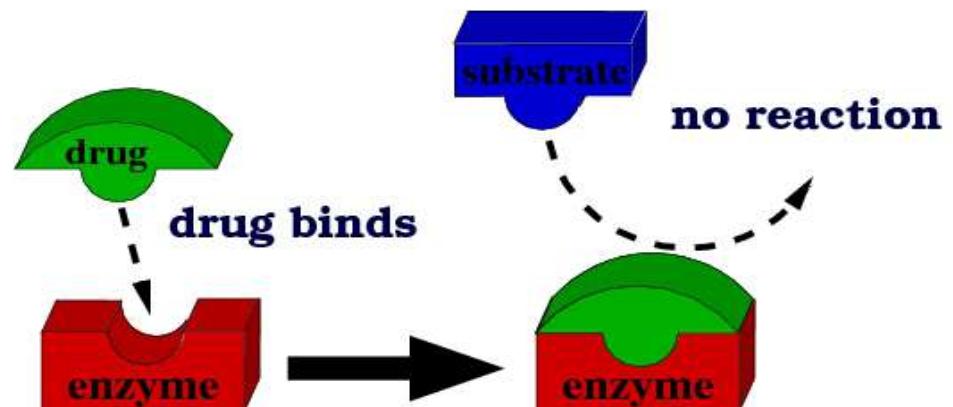
## Drug discovery

Drug design

Ligand database Screening

...

**Drug: active molecule with higher affinity than natural ligand**



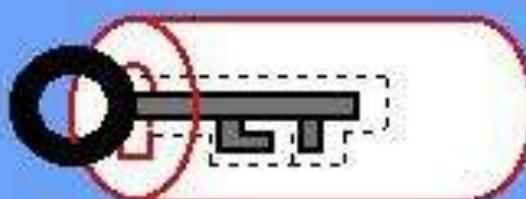
## Lock and Key Analogy



key = substrate



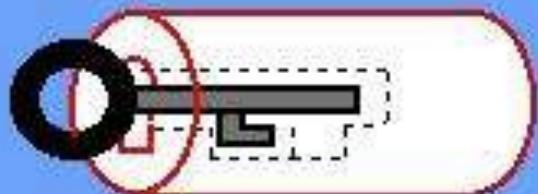
lock = enzyme



correct fit,  
will react



incorrect substrate



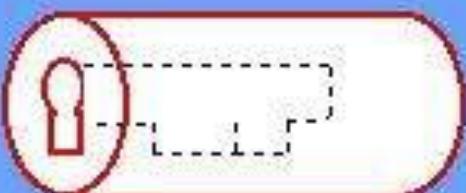
no reaction

C. Ophardt, c. 2003

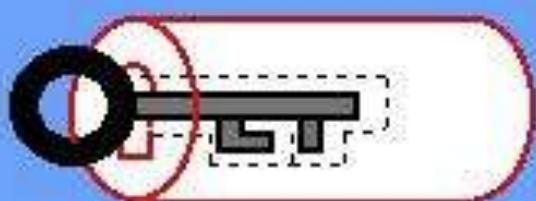
## Lock and Key Analogy



key = substrate



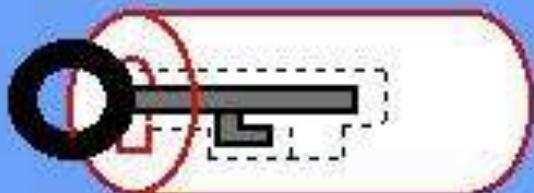
lock = enzyme



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C. Ophardt, c. 2003



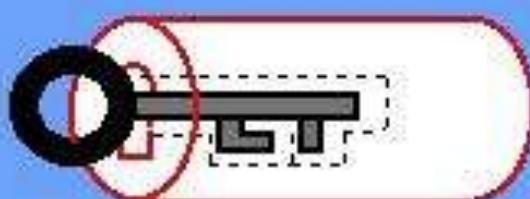
## Lock and Key Analogy



key = substrate



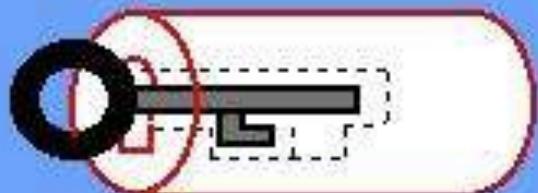
lock = enzyme



correct fit,  
will react



incorrect substrate



no reaction

C. Ophardt, c. 2003



# Methods for ligand database screening:

## Screening in laboratory:

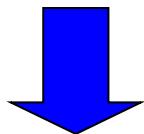
- Automatized,
- but expensive
- and time-consuming



# Methods for ligand database screening:

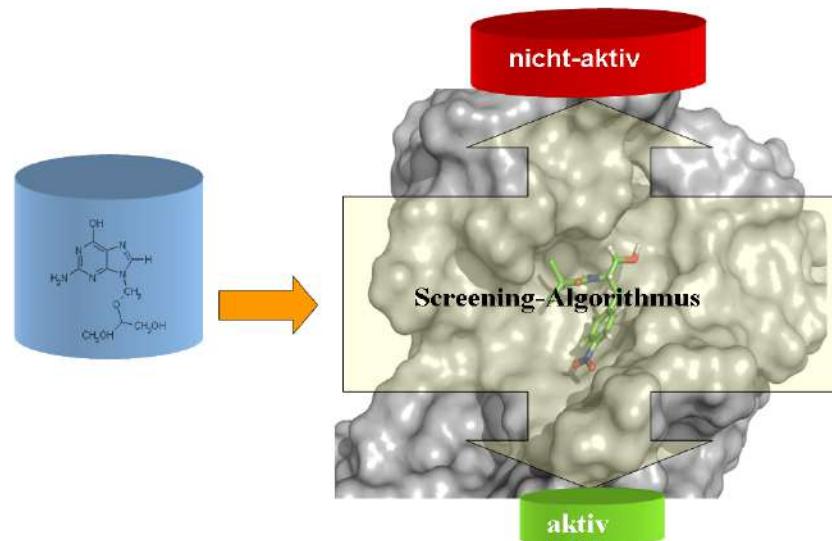
## Screening in laboratory:

- Automatized,
- but expensive
- and time-consuming



## Virtual Screening

- Search for leads
- As pre-stage for exp. tests





## Nobel Prizes and Laureates

Chemistry Prizes ▼ ◀ 2013 ▶

### ▼ About the Nobel Prize in Chemistry 2013

[Summary](#)  
[Prize Announcement](#)  
[Press Release](#)  
[Advanced Information](#)  
[Popular Information](#)  
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[► Martin Karplus](#)  
[► Michael Levitt](#)  
[► Arieh Warshel](#)

[All Nobel Prizes in Chemistry](#)  
[All Nobel Prizes in 2013](#)



### The Nobel Prize in Chemistry 2013

Martin Karplus, Michael Levitt, Arieh Warshel

# The Nobel Prize in Chemistry 2013



© Harvard University  
Martin Karplus



Photo: © S. Fisch  
Michael Levitt



Photo: Wikimedia Commons  
Arieh Warshel

The Nobel Prize in Chemistry 2013 was awarded jointly to Martin Karplus, Michael Levitt and Arieh Warshel "for the development of multiscale models for complex chemical systems".

# Successful Applications of Computer Aided Drug Discovery: Moving Drugs from Concept to the Clinic

Tanaji T. Talele<sup>\*1</sup>, Santosh A. Khedkar<sup>2</sup> and Alan C. Rigby<sup>2</sup>

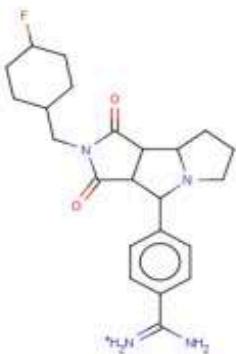
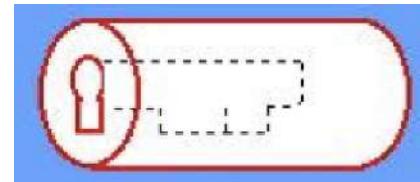
<sup>1</sup>*Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439, <sup>2</sup>Laboratory for Target Validation and Drug Discovery, Center for Vascular Biology Research, Division of Molecular and Vascular Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215*

**Abstract:** Drug discovery and development is an interdisciplinary, expensive and time-consuming process. Scientific advancements during the past two decades have changed the way pharmaceutical research generate novel bioactive molecules. Advances in computational techniques and in parallel hardware support have enabled *in silico* methods, and in particular structure-based drug design method, to speed up new target selection through the identification of hits to the optimization of lead compounds in the drug discovery process. This review is focused on the clinical status of experimental drugs that were discovered and/or optimized using computer-aided drug design. We have provided a historical account detailing the development of 12 small molecules (Captopril, Dorzolamide, Saquinavir, Zanamivir, Oseltamivir, Aliskiren, Boceprevir, Nolatrexed, TMI-005, LY-517717, Rupintrivir and NVP-AUY922) that are in clinical trial or have become approved for therapeutic use.

**Keywords:** Molecular modeling, Structure-activity relationship, X-ray crystallography, Clinical trials, Success stories.



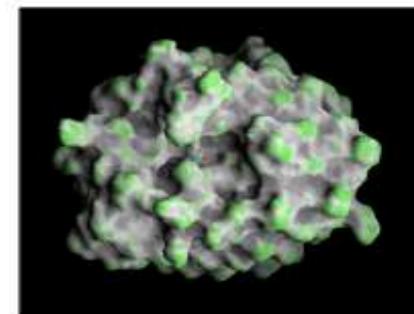
# Virtual Screening



3D Structure of Target

Unknown

Known



Ligand-Based  
Methods

Actives known

Actives **and** inactives known

Similarity  
searching

Pharmacophore  
mapping

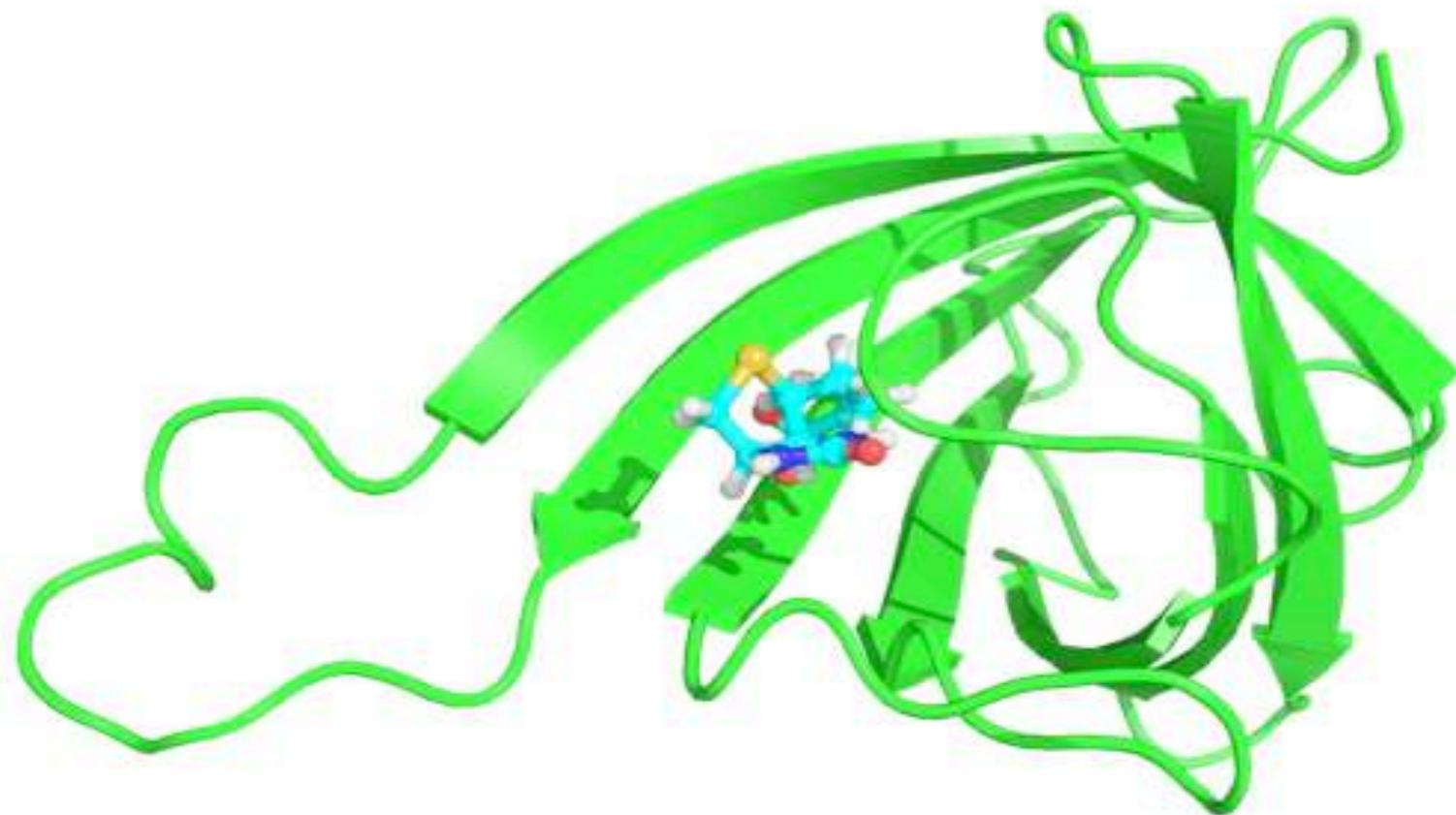
Machine learning  
methods

Structure-Based  
Methods

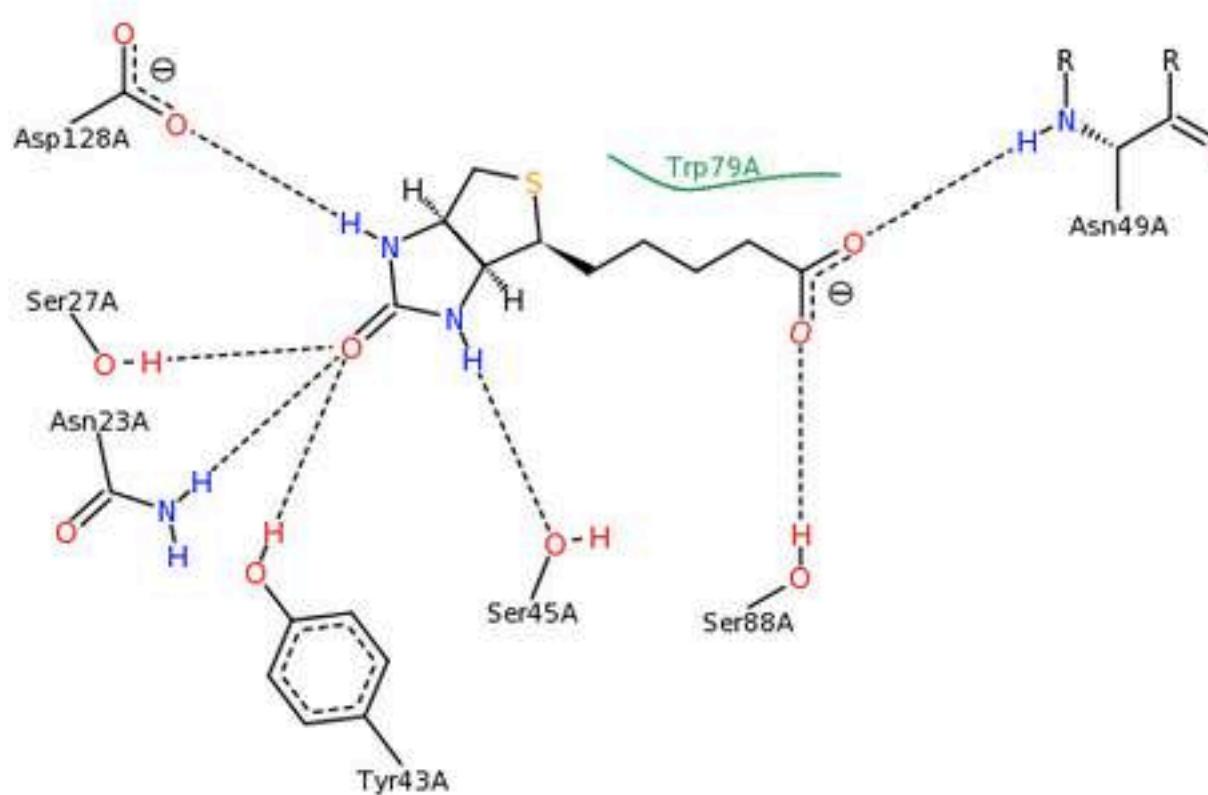
Protein Ligand  
Docking



# MOLECULAR DOCKING SIMULATIONS



# DOCKING PREDICTION AND RESULT EXAMPLE: STREPTAVIDIN-BIOTIN



A docking method must be able to find and reproduce protein-ligand interactions:

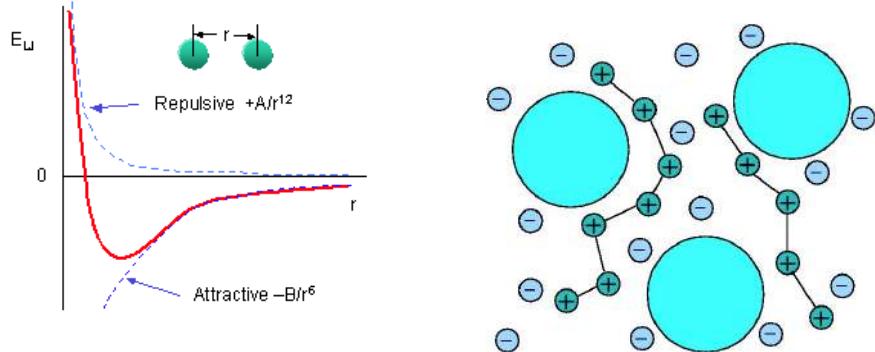
- Electrostatic interactions
- Van der Waals interactions
- Hydrogen bonds
- Lipophilic/Hydrophobic/Solvation
- Many others: aromatic, metals, halogen, etc

# Scoring functions used in Docking

## NON-BONDED INTERACTIONS

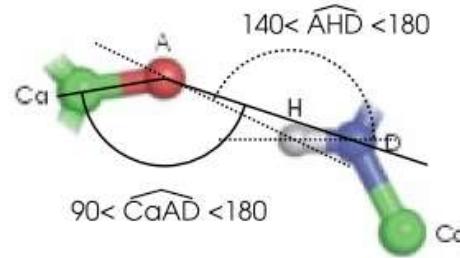
Van der Waals (VDW) + Electrostatics (ES)

$$\sum_{protein \text{ } lig, flSC} \sum \left( \frac{R_{ij}}{r_{ij}^{12}} - \frac{A_{ij}}{r_{ij}^6} + \frac{q_i q_j}{r_{ij}} \right)$$



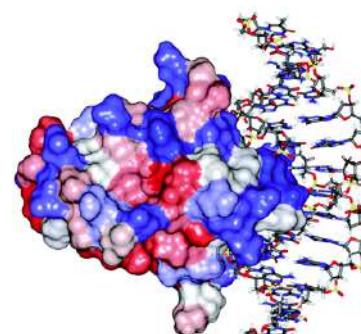
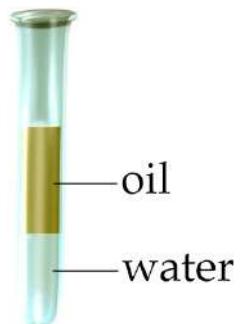
+ Hydrogen Bonds (HBOND)

$$\sum_{h-bonds} \cos \Theta_{ij} \left( \frac{\tilde{R}_{ij}}{r_{ij}^{12}} - \frac{\tilde{A}_{ij}}{r_{ij}^6} \right)$$

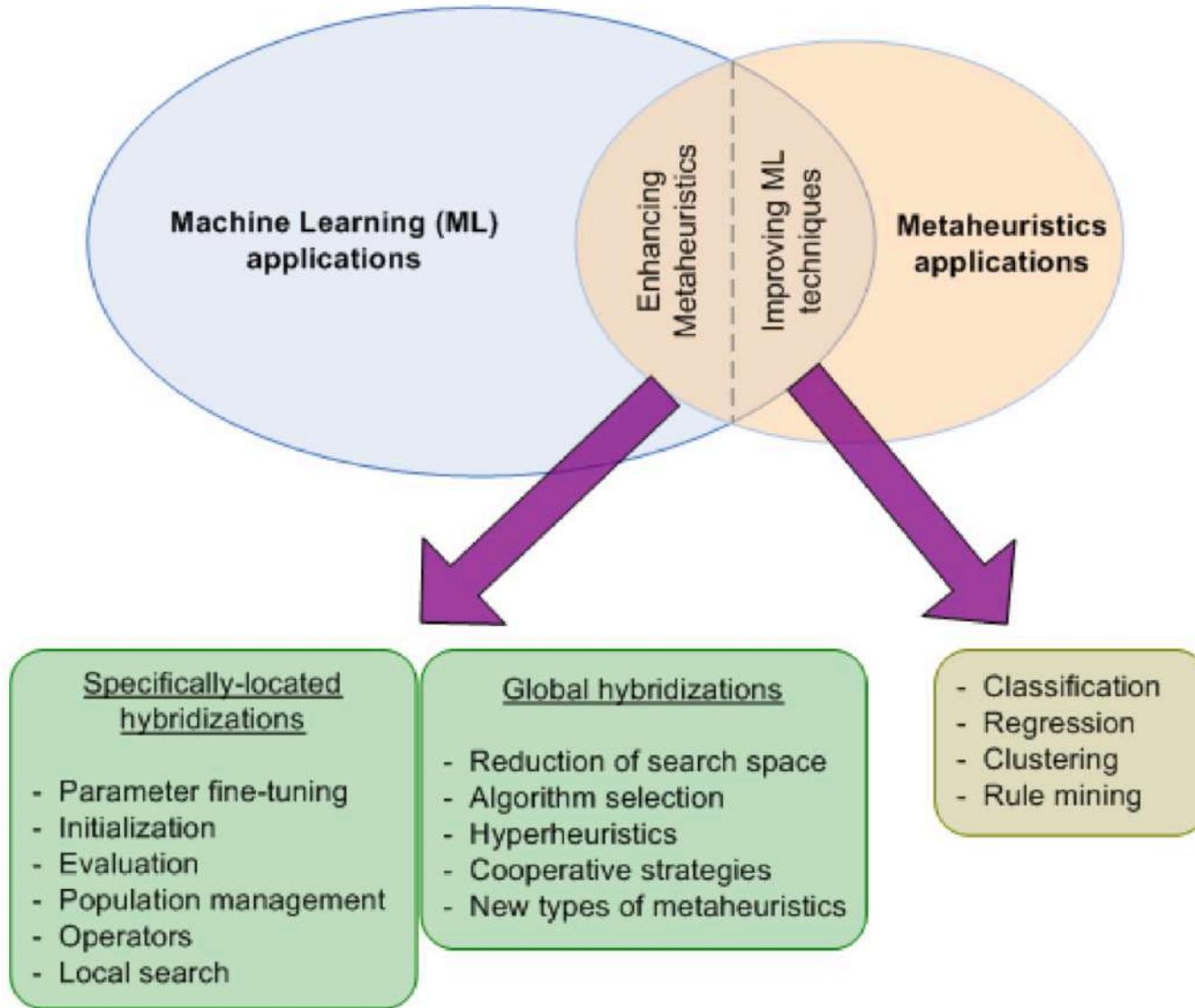


+ Solvation (SASA)

$$\sum_{SASA} \sigma_i A_i$$



# Machine Learning and metaheuristics in Docking



# Bioinformatics and High Performance Computing Research Group

BIOHPC Website

<http://bio-hpc.eu>



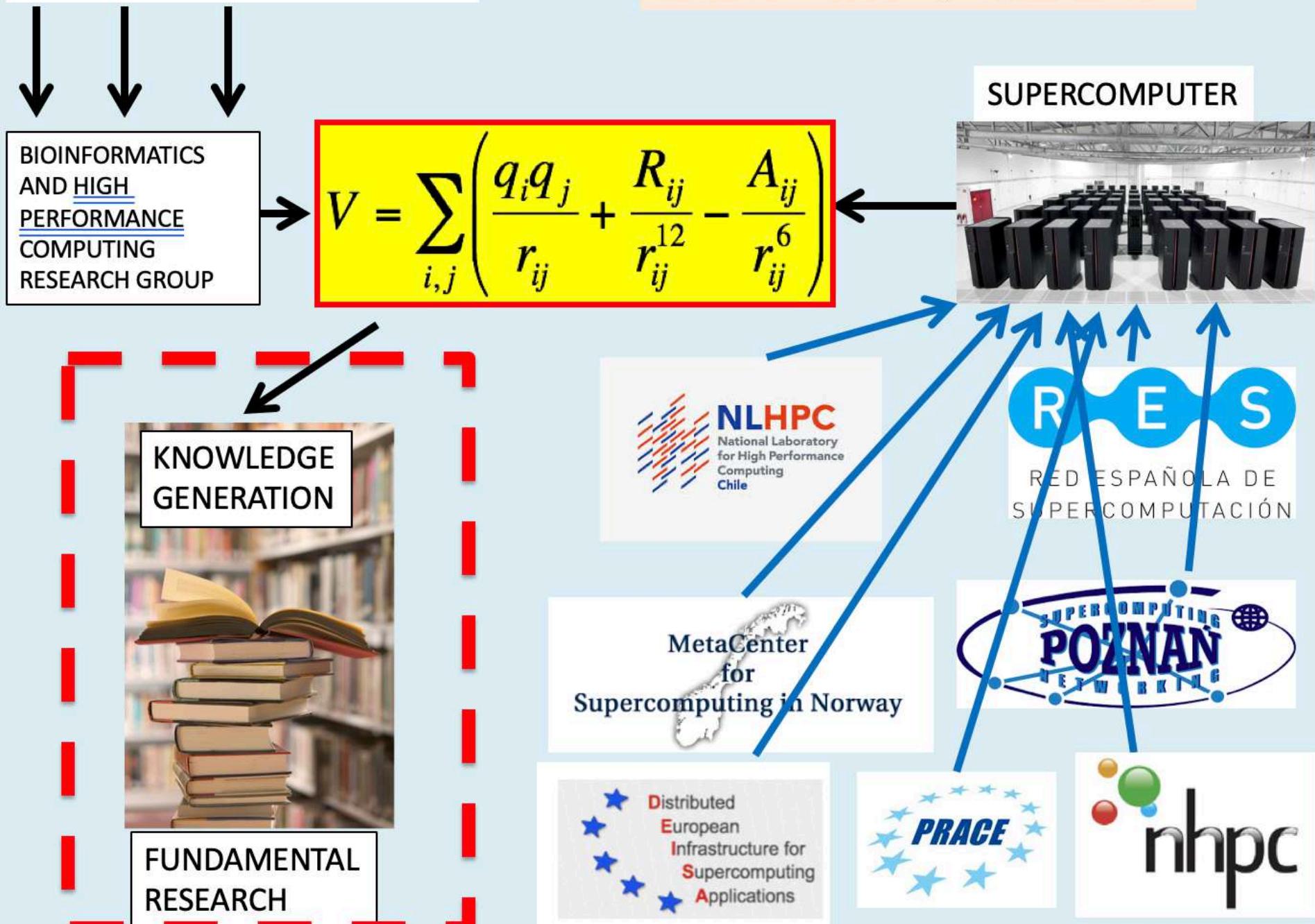
- 2 Full time researchers
- 7 Professors
- 4 PhD Theses defended
- 10 PhD students being supervised
- Scientific output since May 2013
  - Journals: 80
  - Conference proceedings: 36
  - Contracts: 4
  - Projects (PI): 14
  - Funding attracted: ca. 600.000€
- NVIDIA Teaching and Research Center

## TECHNOLOGY TRANSFER

- 3 international patents (licensed)
- Nanomatch.de
- Talentum-biotech.com
- Blind Docking Server
- Md.use
- 2016 HiPEAC Tech Transfer Award
- Participation in CDTI projects

€€€, RESEARCH FUNDS (UCAM)

OUR "STARTUP" / "COMPANY"



# Blue Gene



El sistema  
El armario (rack)  
32 nodos  
[4096 cores]



64 racks [262.144 cores]

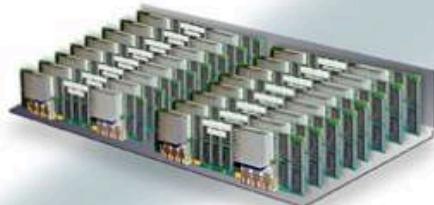


2.62 PFLOPS

El nodo

16 placas [128 cores]

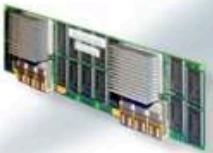
40960 GFLOPS



Las placas base

2 chips [8 cores]

1280 GFLOPS



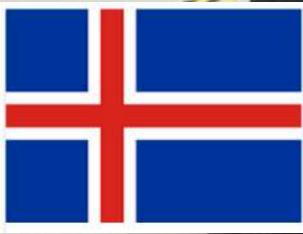
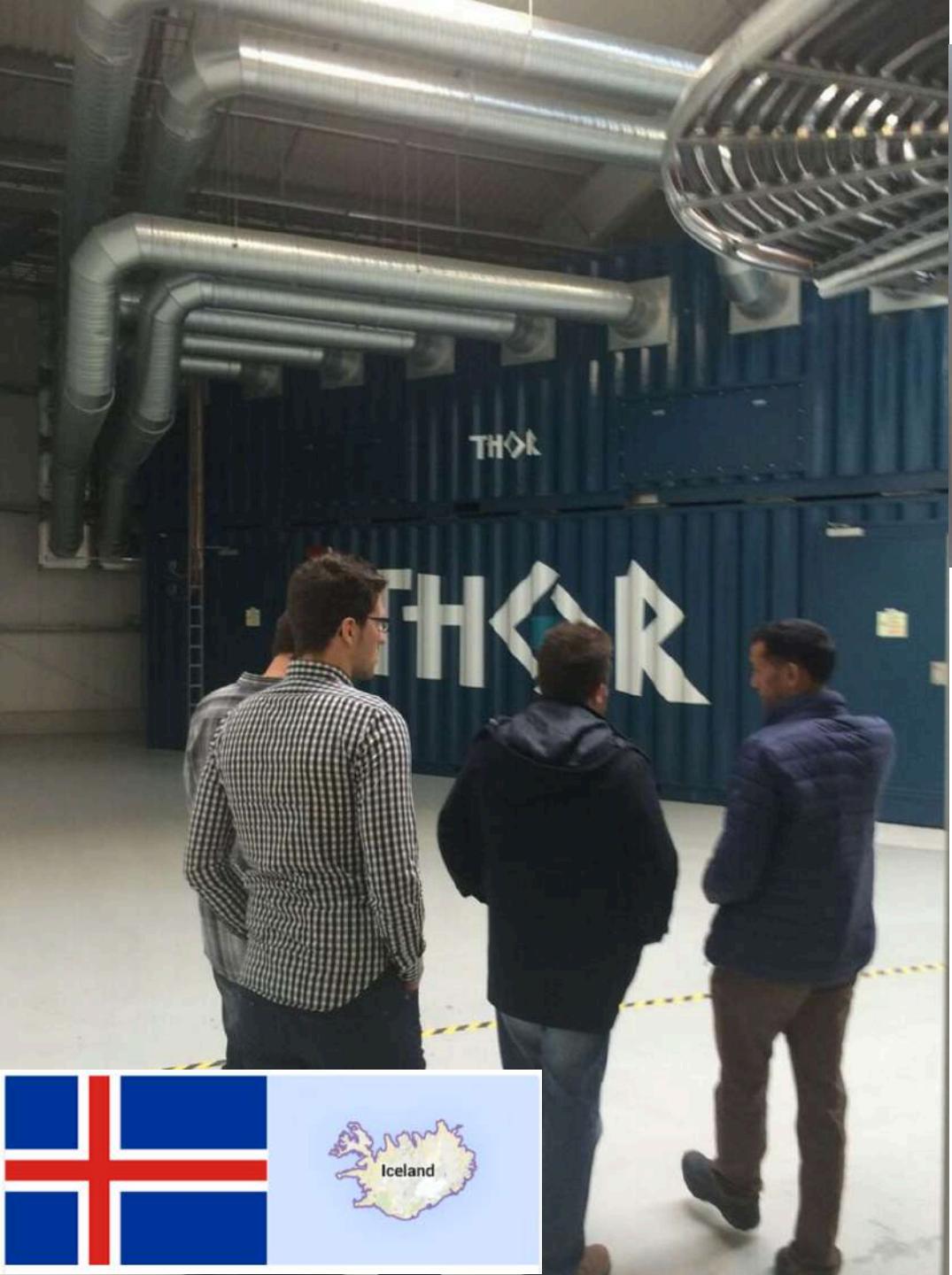
80 GFLOPS

El chip  
4 cores

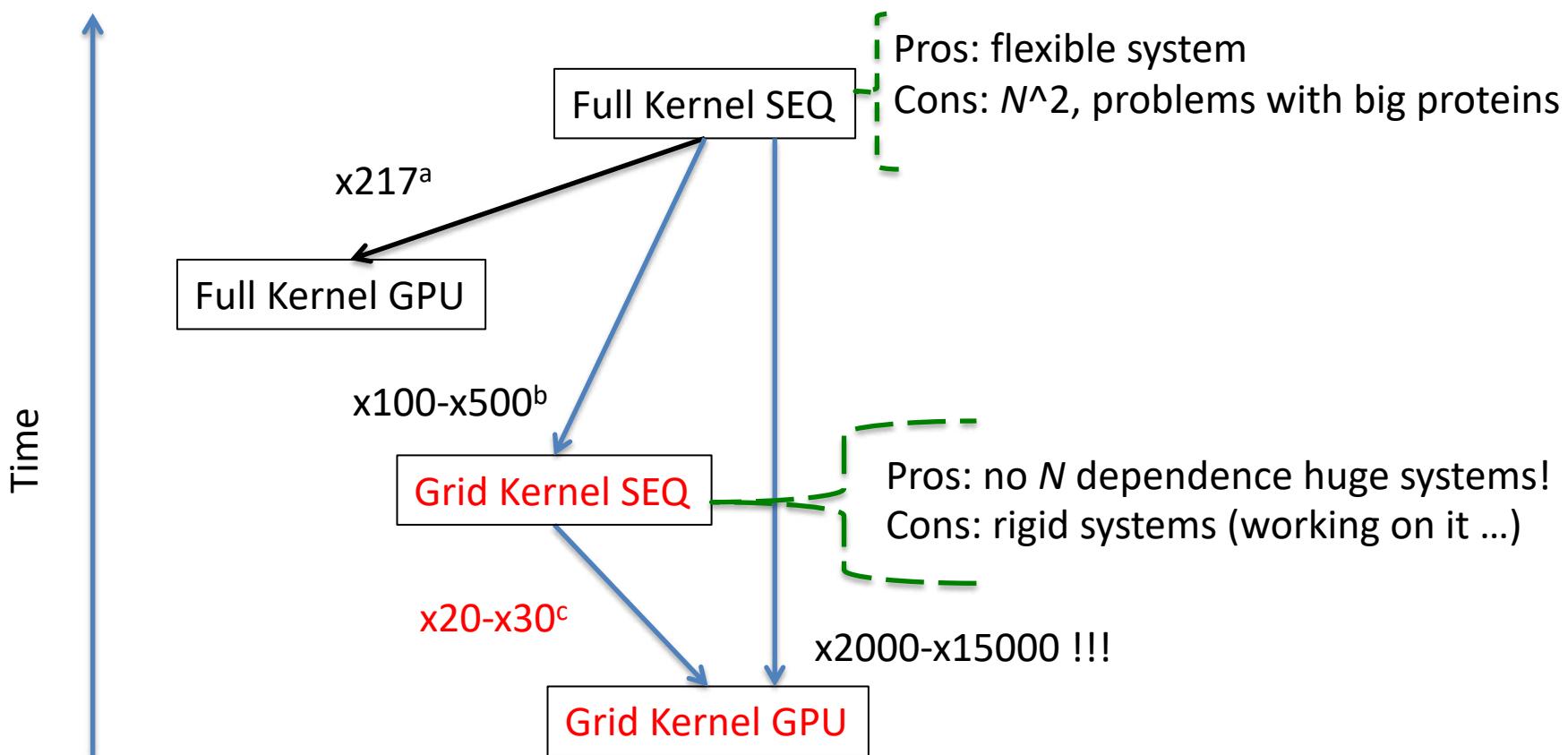


40 GFLOPS

From Desktop PC (40GFlops) to Supercomputer (2.62PFlops)  
→ X 65500 !!!



## Non-bonded interactions using Full and Grid Kernels



<sup>a</sup> Ginés Guerrero, Horacio Pérez-Sánchez, Wolfgang Wenzel, José Cecilia, and José García. Effective parallelization of non-bonded interactions kernel for virtual screening on GPUs. In *5th International Conference on Practical Applications of Computational Biology; Bioinformatics* (PACBB 2011), volume 93, pages 63–69

<sup>b</sup> EC Meng, BK Shoichet, and ID Kuntz. Automated Docking with Grid-Based Energy Evaluation. *Journal of Computational Chemistry*, 13(4):505–524, 1992.

<sup>c</sup> I. Sánchez-Linares, H. Pérez-Sánchez, J.M. García, (2011) “Accelerating Grid Kernels for Virtual Screening on Graphics Processing Units” In: Proceedings of the International Conference on Parallel Computing (Parco) Conference on Parallel Computing (ParCo).

# METADOCK: A parallel metaheuristic schema for virtual screening methods

Baldomero Imbernón<sup>1</sup>, José M Cecilia<sup>1</sup>, Horacio Pérez-Sánchez<sup>1</sup> and Domingo Giménez<sup>2</sup>

The International Journal of High Performance Computing Applications

1–15

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journals.sagepub.com/home/hpc



## Abstract

Virtual screening through molecular docking can be translated into an optimization problem, which can be tackled with metaheuristic methods. The interaction between two chemical compounds (typically a protein, enzyme or receptor, and a

- Multicore / Manycore / Multi-GPU / rCUDA
- Tunable Scoring Function (SF) and Metaheuristics
  - Includes SF clon of AD4
    - > x20 speedup over Autodock 4 (AD4)
    - Increased accuracy ( $\text{RMSD} < 4$ ) over DUD benchmark using AD4 SF Clon vs AD4 ( $\text{RMSD} < 9$ )
  - Implements **BLIND DOCKING** technique

## Keywords

Drug discovery, virtual screening, molecular docking, high performance computing, metaheuristics, heterogeneous computing

# METADOCK 2: A high-throughput parallel metaheuristic scheme for molecular docking

Baldomero Imbernón<sup>1,\*</sup>, Antonio Serrano<sup>1</sup>, Andrés Bueno-Crespo<sup>1</sup>, José L. Abellán<sup>1</sup>, Horacio Pérez-Sánchez<sup>1,\*</sup> and José M. Cecilia<sup>1,\*</sup>

<sup>1</sup> Structural Bioinformatics and High Performance Computing Research Group (BIO-HPC), Computer Engineering Department, Universidad Católica de Murcia (UCAM), 30107, Murcia, Spain

\* To whom correspondence should be addressed.

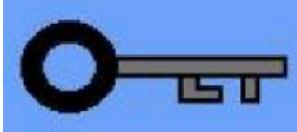
Associate Editor:

Received on XXXXX; revised on XXXXX; accepted on XXXXX

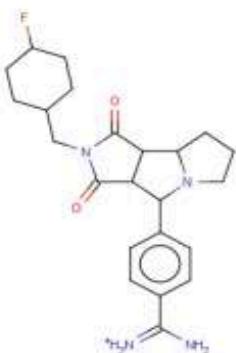
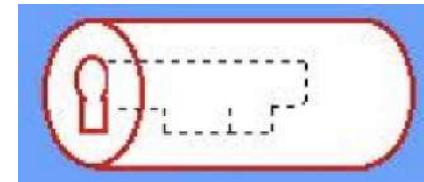
## Abstract

**Motivation:** Molecular docking methods are extensively used to predict the interaction between protein-ligand systems in terms of structure and binding affinity, through the optimization of a physics-based scoring function. However, the computational requirements of these simulations grow exponentially with: (1) the global optimization procedure, (2) the number and degrees of freedom of molecular conformations generated, and (3) the mathematical complexity of the scoring function.

**Results:** In this work we introduce a novel molecular docking method named *METADOCK 2*, which incorporates several novel features such as (1) a ligand-dependent blind docking approach that exhaustively scans the whole protein surface to detect novel allosteric sites, (2) an optimization method to enable the use of a wide branch of metaheuristics, and (3) a heterogeneous implementation based on multicore CPUs and multiple Graphics Processing Units (GPUs). Two representative scoring functions implemented in *METADOCK 2* are extensively evaluated in terms of computational performance and accuracy using several benchmarks (such as the well-known DUD) against AutoDock 4.2 and AutoDock Vina. Results place *METADOCK 2* as an efficient and accurate docking methodology able to deal with complex systems where computational demands are staggering and which outperforms both AutoDock Vina and AutoDock 4.



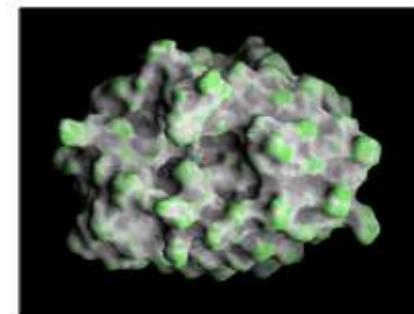
# Virtual Screening



3D Structure of Target

Unknown

Known



Ligand-Based  
Methods

Actives known

Actives **and** inactives known

Structure-Based  
Methods

Similarity  
searching

Pharmacophore  
mapping

Machine learning  
methods

Protein Ligand  
Docking



# SCIENTIFIC REPORTS



OPEN

## OptiPharm: An evolutionary algorithm to compare shape similarity

Received: 18 July 2018

Accepted: 11 December 2018

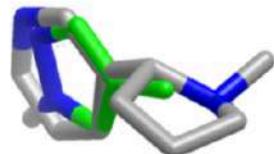
Published online: 04 February 2019

S. Puertas-Martín<sup>1,3</sup>, J. L. Redondo<sup>1</sup>, P. M. Ortigosa<sup>1</sup> & H. Pérez-Sánchez<sup>2</sup>

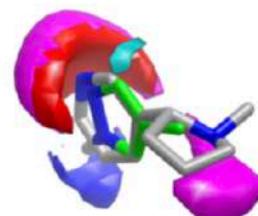
Virtual Screening (VS) methods can drastically accelerate global drug discovery processes. Among the most widely used VS approaches, Shape Similarity Methods compare in detail the global shape of a query molecule against a large database of potential drug compounds. Even so, the databases are so enormously large that, in order to save time, the current VS methods are not exhaustive, but they are mainly local optimizers that can easily be entrapped in local optima. It means that they discard promising compounds or yield erroneous signals. In this work, we propose the use of efficient global optimization techniques, as a way to increase the quality of the provided solutions. In particular, we introduce OptiPharm, which is a parameterizable metaheuristic that improves prediction accuracy and offers greater computational performance than WEGA, a Gaussian-based shape similarity method. OptiPharm includes mechanisms to balance between exploration and exploitation to quickly identify regions in the search space with high-quality solutions and avoid wasting time in non-promising areas. OptiPharm is available upon request via email.

Query compound: DB01213

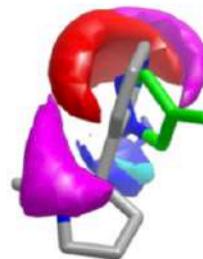
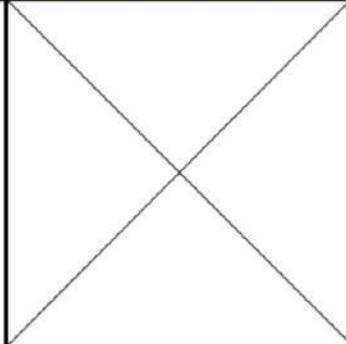
LBVS-S. Best compound: DB00184



$$Tc_S = 0.621$$

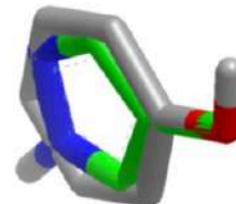


$$Tc_E^{Eval} = 0.500$$

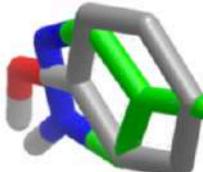


$$Tc_E = 0.609$$

LBVS-EP. Best compound: DB03255



$$Tc_S = 0.963$$

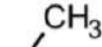


$$Tc_S^{Eval} = 0.880$$

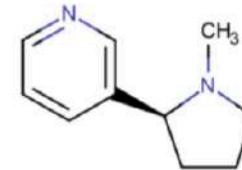


$$Tc_E = 0.810$$

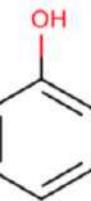
QUERY



**DB01213**



**DB00184**



**DB03255**

Figure 4: Results of LBVS-S and LBVS-E where  $Query = DB01213$ . The Query compound is colored green. Query electrostatic fields are colored deep blue and red. Best compounds are shown in grey and their electrostatic potential fields in light blue and pink.



# BRUSELAS: HPC Generic and Customizable Software Architecture for 3D Ligand-Based Virtual Screening of Large Molecular Databases

Antonio J. Banegas-Luna,<sup>\*,†</sup> José P. Cerón-Carrasco,<sup>†</sup> Savíns Puertas-Martín,<sup>‡</sup> and Horacio Pérez-Sánchez<sup>\*,†</sup>

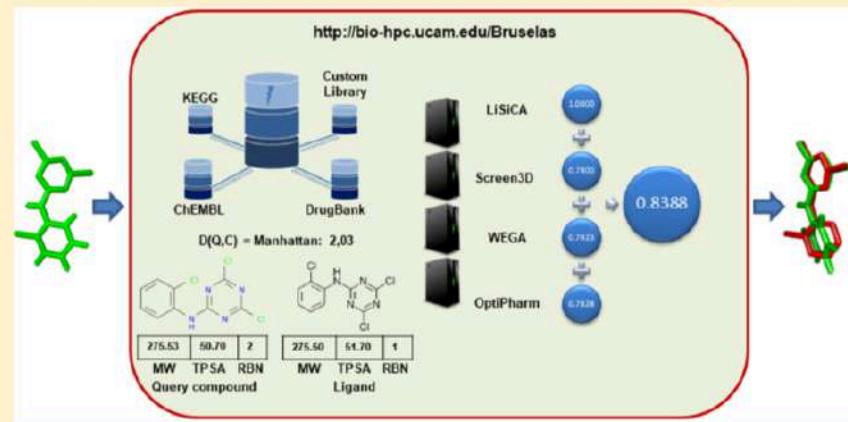
<sup>†</sup>Bioinformatics and High Performance Computing Research Group (BIO-HPC), Computer Engineering Department, Universidad Católica San Antonio de Murcia (UCAM), Campus de los Jerónimos s/n, 30107 Murcia, Spain

<sup>‡</sup>Supercomputing - Algorithms Research Group (SAL), Department of Informatics, University of Almería, Agrifood Campus of International Excellence, ceiA3, Almería, 04120, Spain

<http://bio-hpc.eu/software/Bruselas/>

Supporting Information

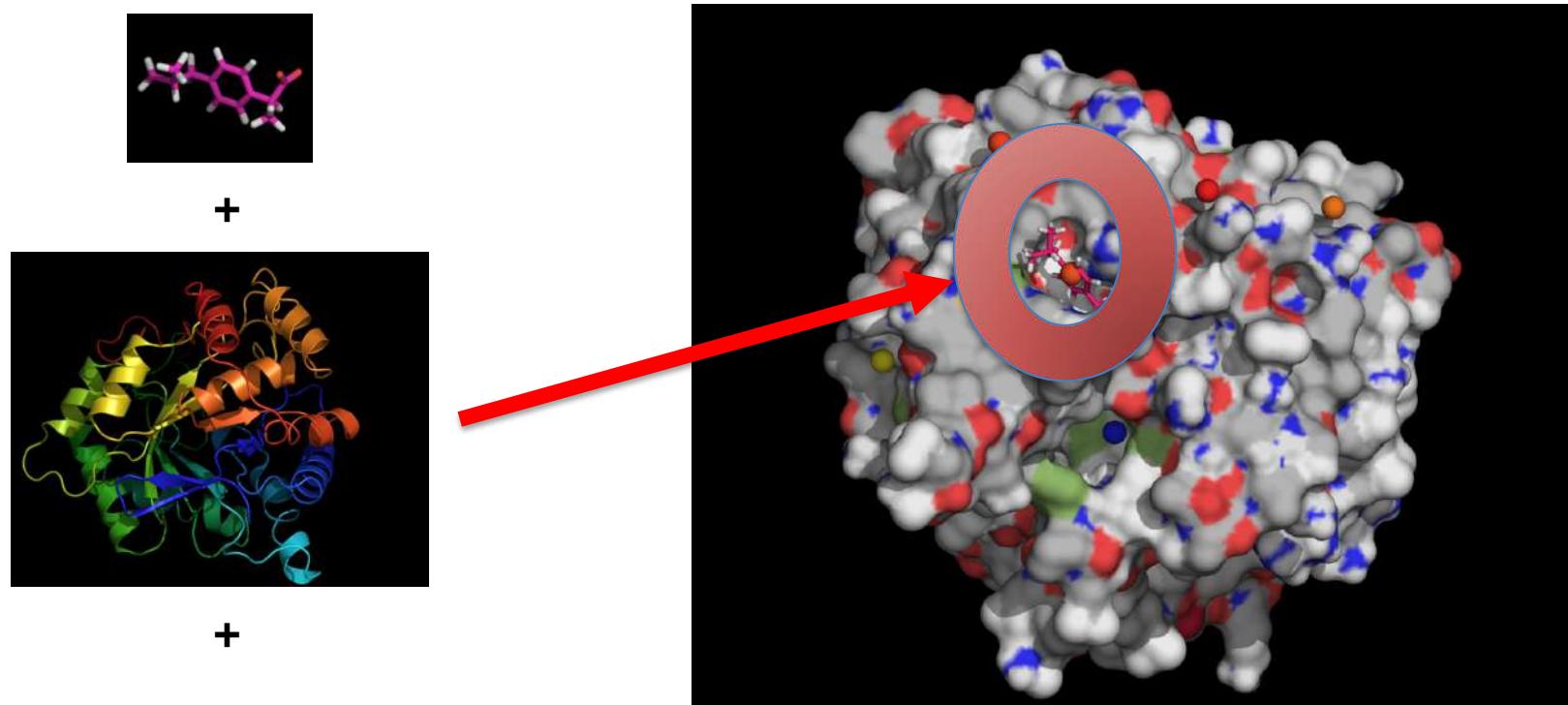
**ABSTRACT:** BRUSELAS (balanced rapid and unrestricted server for extensive ligand-aimed screening) is a novel, highly efficient web software architecture for 3D shape and pharmacophore searches in *off the cuff* libraries. A wide panel of shape and pharmacophore similarity algorithms are combined to avoid unbiased results while yielding consensus scoring functions. To evaluate its reliability, BRUSELAS was tested against other similar servers (e.g., USR-VS, SwissSimilarity, ChemMapper) to search for potential antidiabetic drugs. A web tool is developed for users to customize their tasks and is accessible free of any charge or login at <http://bio-hpc.eu/software/Bruselas>. Source code is available on request.



# OBTAINED HPC IMPROVEMENTS: NOT JUST SPEEDUP BUT NOVEL DEVELOPMENTS

## “CLASSICAL” Docking:

Calculating interaction between a small molecule (drug leads) and a specific location of a protein.

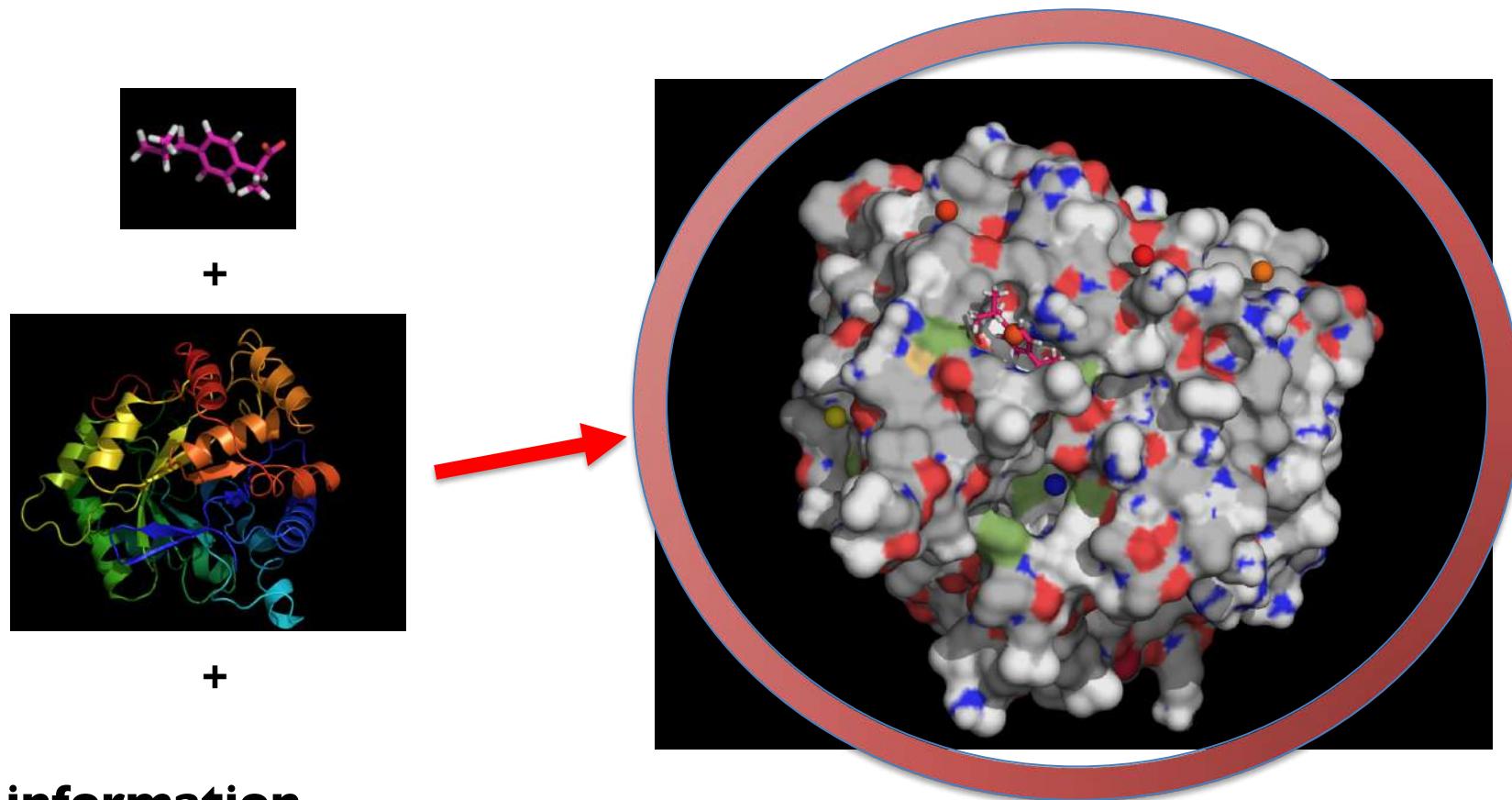


## Specific Coordinates

# OBTAINED HPC IMPROVEMENTS: NOT JUST SPEEDUP BUT NOVEL DEVELOPMENTS

## BLIND Docking

Calculating interaction between a small molecule (drug leads) and **WHOLE** protein



**No information  
about specific  
interaction area needed**



**Discovery of unexpected / unexplored  
interaction sites  
and novel chemical scaffolds / drugs**

# Blind Docking Server

- Available at:
  - <https://bio-hpc.ucam.edu/achilles> **DEMO**
- Not available in free or commercial computational chemistry packages (Maestro, Schrödinger), although requested by users
- Benchmarked against other state of the art methods
- Interest shown from some companies (advanced versions)
- Since 2017 it has been used in more than 30 publications from users in high impact factor journals and main paper not published (yet)

# MCC950 closes the active conformation of NLRP3 to an inactive state

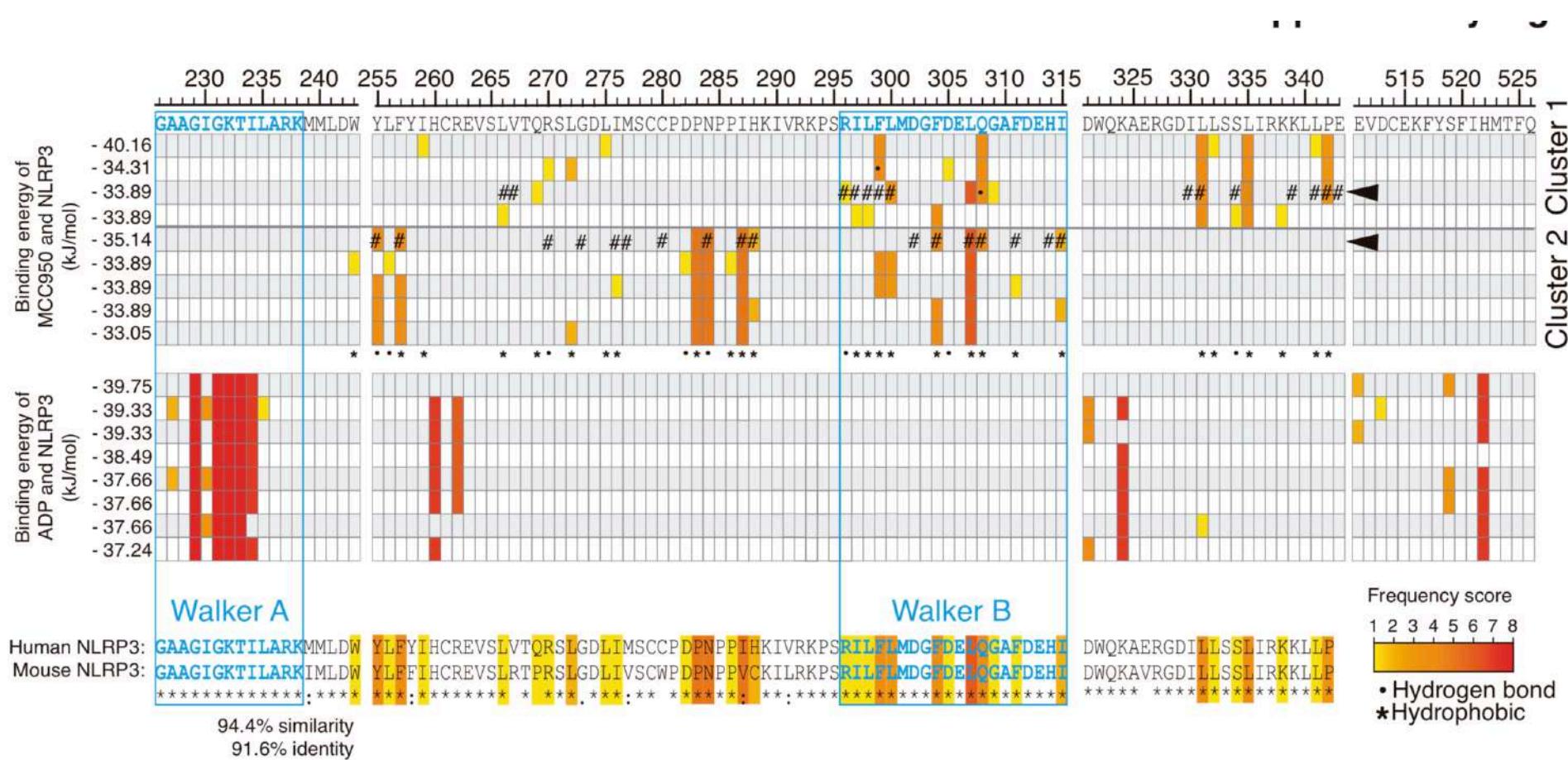
Ana Tapia-Abellán<sup>1</sup>, Diego Angosto-Bazarrá<sup>1</sup>, Helios Martínez-Banaclocha<sup>1</sup>, Carlos de Torre-Minguela<sup>1</sup>, Jose P. Cerón-Carrasco<sup>2</sup>, Horacio Pérez-Sánchez<sup>2</sup>, Juan I. Arostegui<sup>3</sup> and Pablo Pelegrin  <sup>1\*</sup>

**NLRP3 (NOD-like receptor pyrin domain-containing protein 3) is an innate immune sensor that contributes to the development of different diseases, including monogenic autoinflammatory syndromes, gout, atherosclerosis, and Alzheimer's disease. The molecule sulfonylurea MCC950 is a NLRP3 inflammasome inhibitor with potential clinical utility. However, the mechanism of action of MCC950 remains unknown. Here, we characterize the mechanism of action of MCC950 in both wild-type and autoinflammatory-related NLRP3 mutants, and demonstrate that MCC950 closes the 'open' conformation of active NLRP3.**

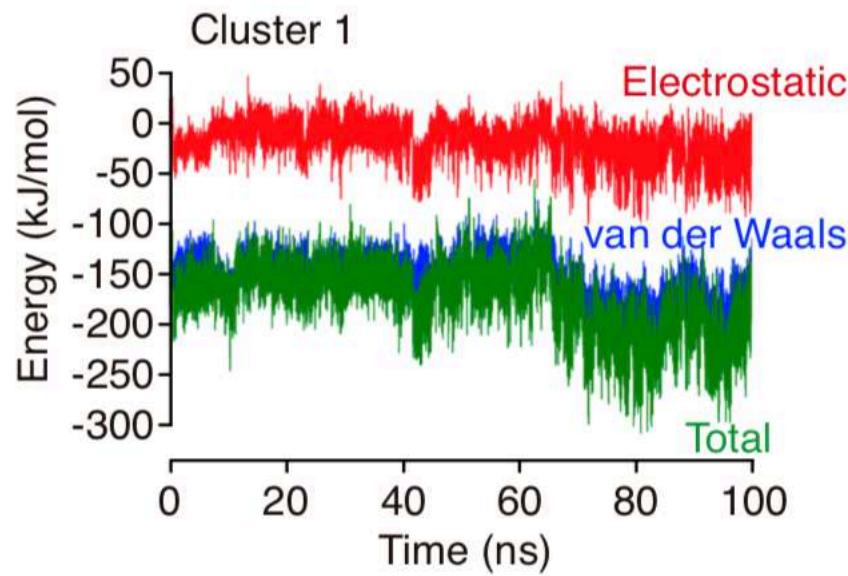
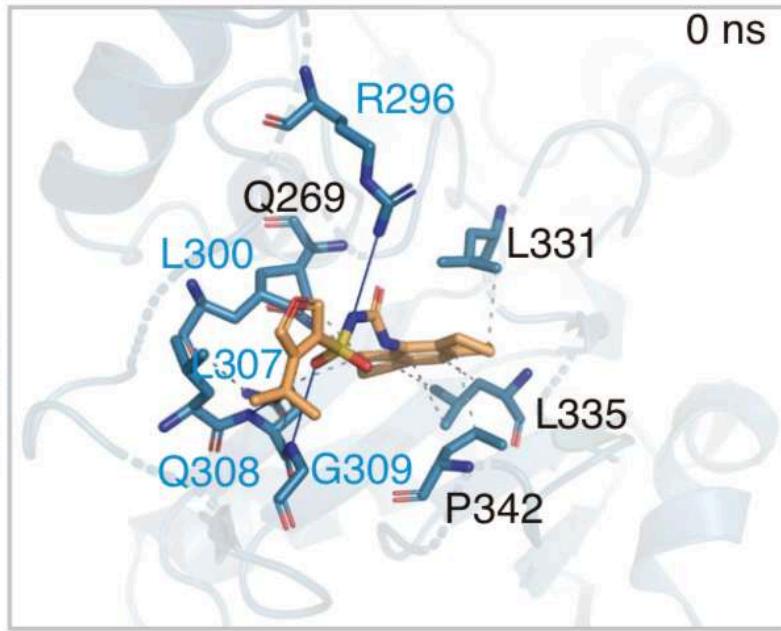
due to variations in protein expression (Supplementary Fig. 1c,d) and was also observed in other pathological mutants of NLRP3 (Supplementary Fig. 1c). NLRP3 BRET signal was intramolecular, as a stable signal was found when increased concentrations of the sensor was expressed (Supplementary Fig. 1b). The incubation of cells expressing the NLRP3 p.D305N BRET sensor with MCC950 at different doses or over increasing times, resulted in an increase of the BRET signal (Fig. 1a,b). These results suggest that MCC950 induces closure of the active NLRP3 p.D305N conformation without changing YFP signals (Supplementary Fig. 1e). MCC950 increased BRET signal from different NLRP3 pathologi-

**Top selected application of the Blind Docking technique by our group**

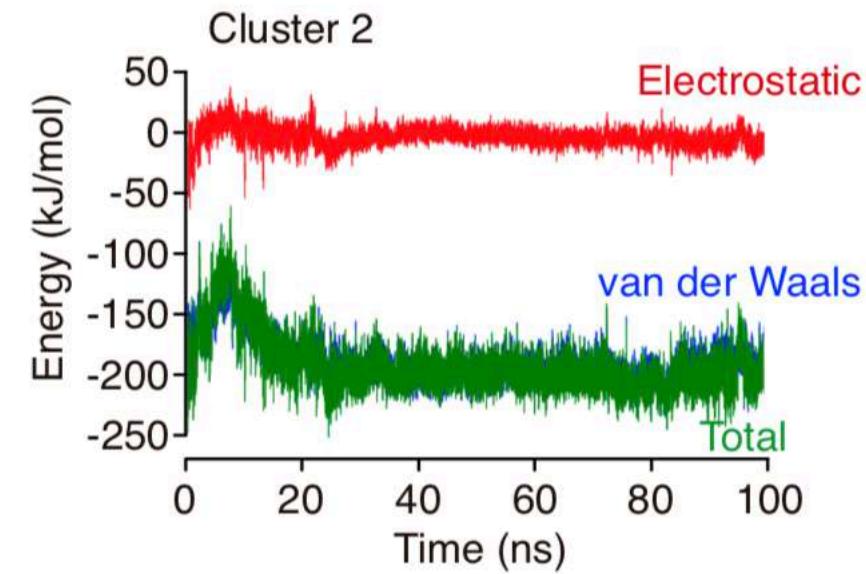
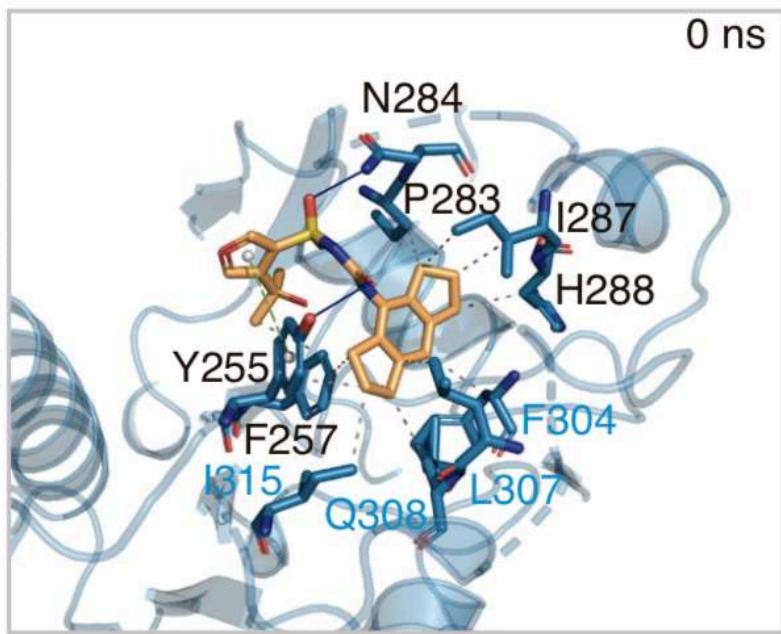
# MCC950 closes the active conformation of NLRP3 to an inactive state



Cluster 1

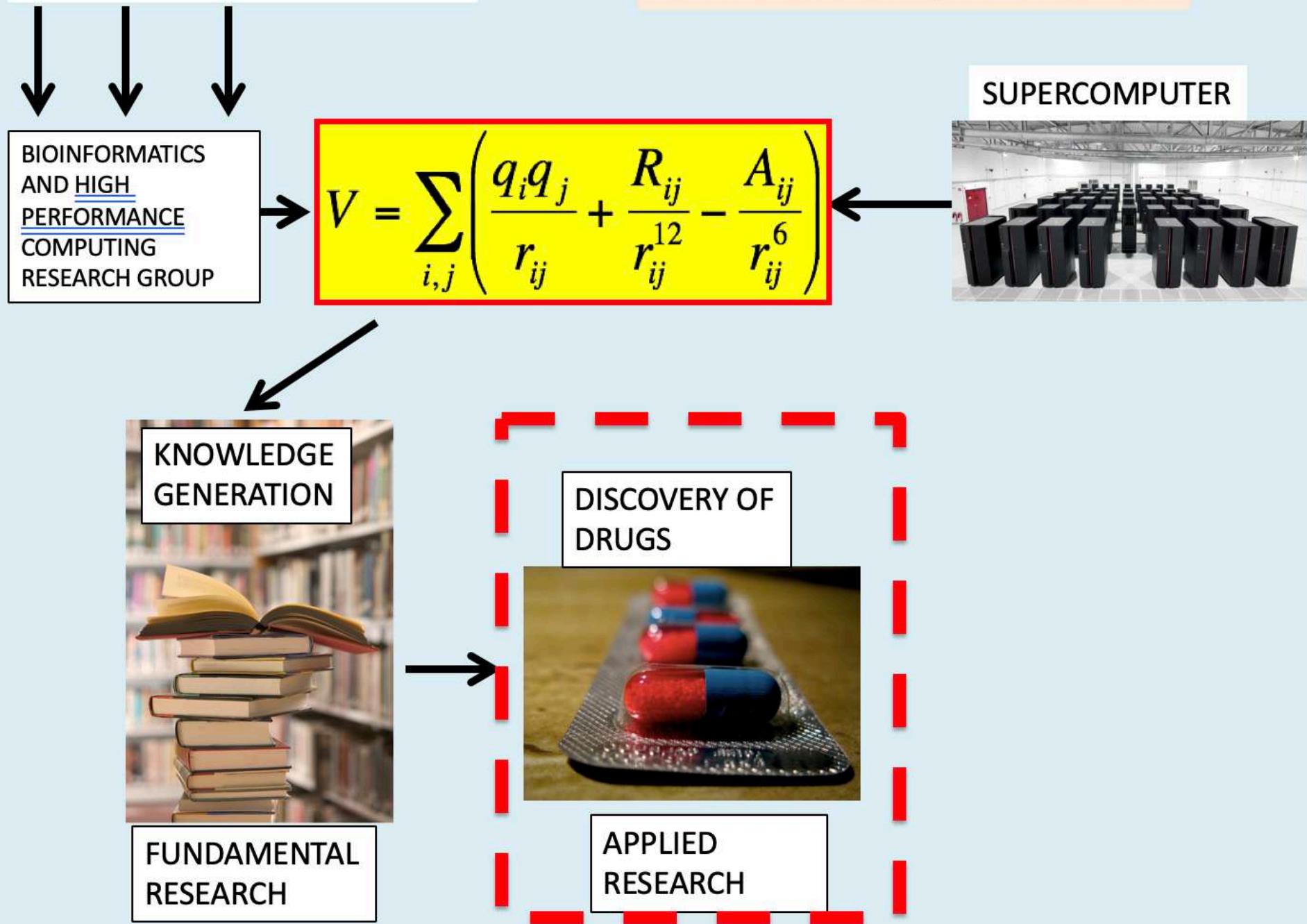


Cluster 2



€€€, RESEARCH FUNDS (UCAM)

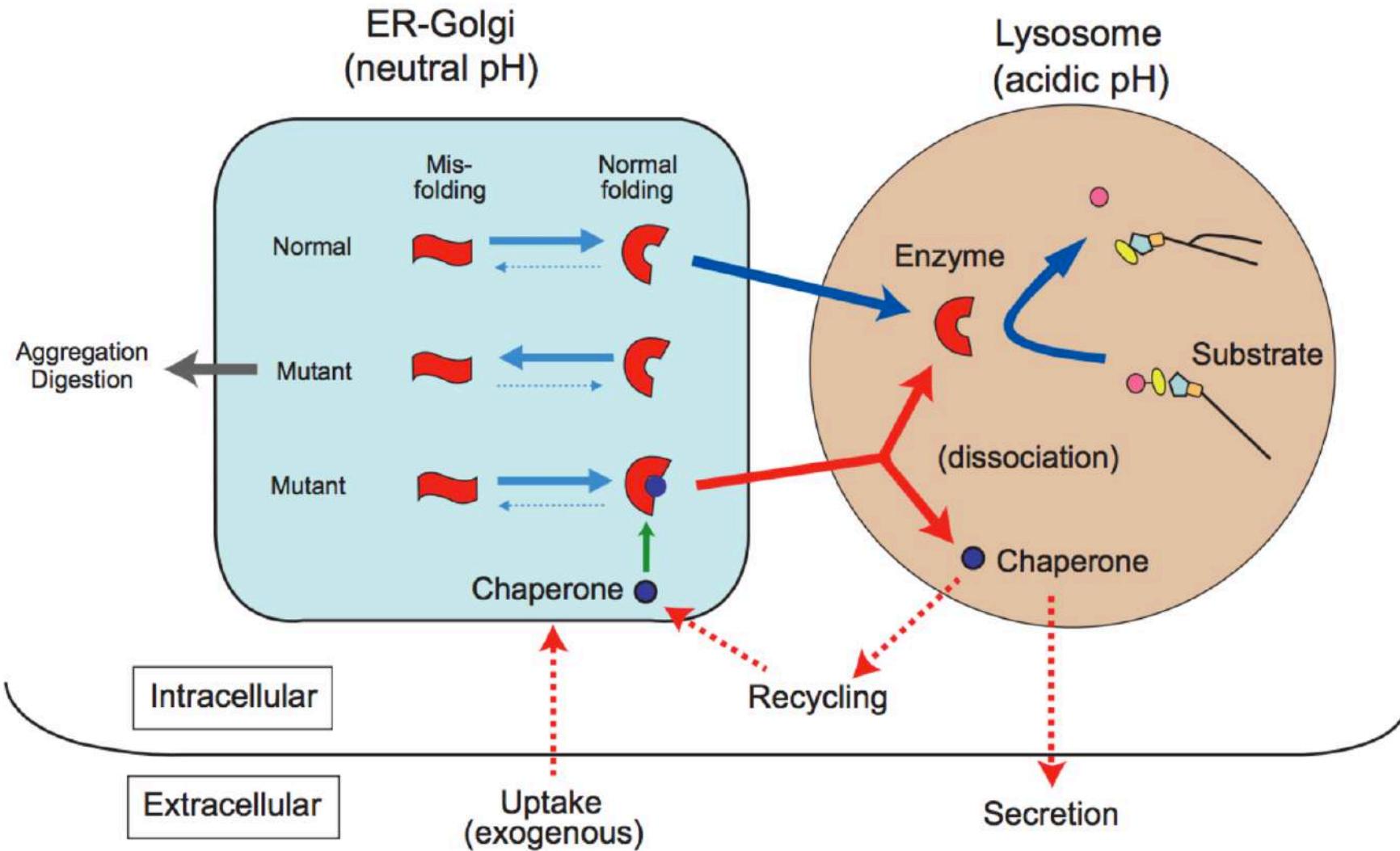
## OUR "STARTUP" / "COMPANY"



# BIO-HPC research group Drug Discovery pipeline

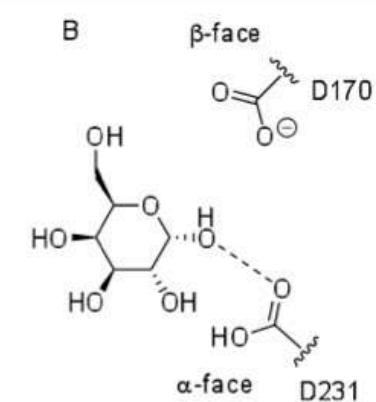
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Colorectal cancer	FDA 1				Requested	Patent filed
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	Merck compound				Requested	Patent filling in process
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Lipase inhibitor	FDA					
Fabry disease	Chemical safe compound					Paper published
Anticoagulants	Human endogenous compound				Requested	Patented and published
Inflamasoma	FDA				In process	
Antibacterials in odontology	FDA				In process	
Xylella fastidiosa	Natural compounds				In process	





Suzuki, Yoshiyuki, Seiichiro Ogawa, and Yasubumi Sakakibara. "Chaperone therapy for neuronopathic lysosomal diseases: competitive inhibitors as chemical chaperones for enhancement of mutant enzyme activities." *Perspectives in medicinal chemistry* 3 (2009): 7.

# MOLECULAR CHAPERONES AND FABRY DISEASE STATE OF THE ART, CHEMICAL OPTIMIZATION



# MOLECULAR CHAPERONES AND FABRY DISEASE STATE OF THE ART, CHEMICAL OPTIMIZATION

ACS Chemical Biology

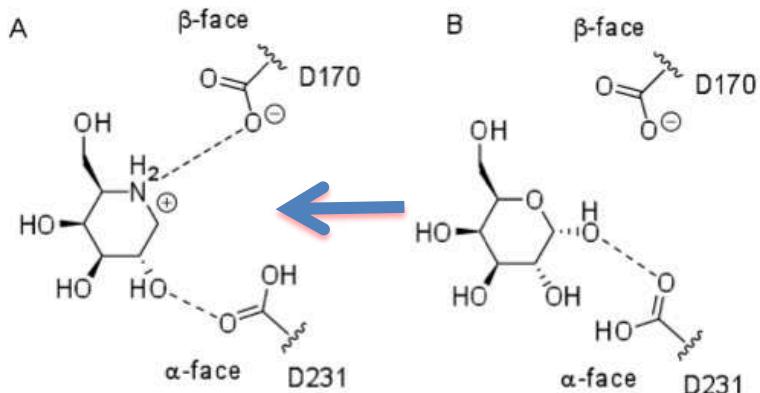


Figure 1. Design criteria for DGJ-ArTs as PCs for FD. Key hydrogen-bonding interactions i (D231) residues in the complexes of  $\alpha$ -Gal A with DGJ (A) and D-galactose (B) and chaperones.

DGJ → 100000x increase in affinity

## MIGALASTAT

# MOLECULAR CHAPERONES AND FABRY DISEASE STATE OF THE ART, CHEMICAL OPTIMIZATION

ACS Chemical Biology

Articles

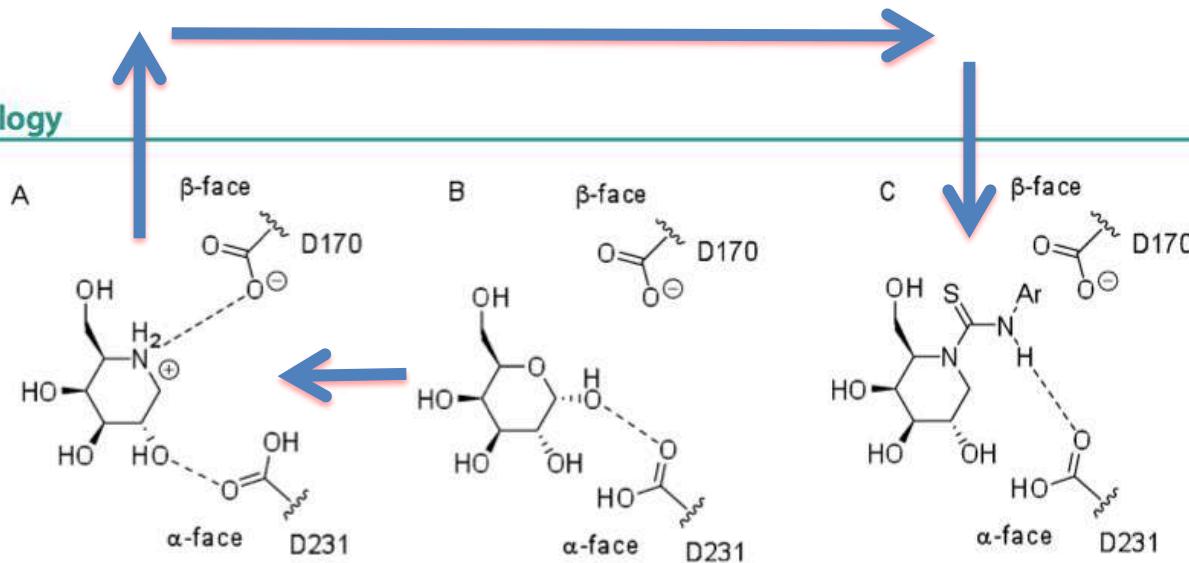


Figure 1. Design criteria for DGJ-ArTs as PCs for FD. Key hydrogen-bonding interactions involving the catalytic aspartate (D170) and aspartic acid (D231) residues in the complexes of  $\alpha$ -Gal A with DGJ (A) and d-galactose (B) and expected scenario for DGJ-ArT (C) pharmacological chaperones.

DGJ-ArT → new family of chaperones

PROBLEM: interference with enzymatic activity

OUR PROPOSAL: discover molecules that stabilize enzyme but do not interact with active site

Yu, Y., Mena-Barragán, T., Higaki, K., Johnson, J. L., Drury, J. E., Lieberman, R. L., et al. (2014). Molecular Basis of 1-Deoxygalactonojirimycin Arylthiourea Binding to Human  $\alpha$ -Galactosidase A: Pharmacological Chaperoning Efficacy on Fabry Disease Mutants. *ACS Chemical Biology*, 140512093218005. doi:10.1021/cb500143h

# COLLABORATION WITH UNIVERSITY OF NAPLES



HOME | ABOUT US » | IRDiRC ACTIVITIES » | RARE DISEASES RESEARCH » | REPORTS & GUIDELINES » | USEFUL LINKS » | IRDiRC PRIVATE WEBSITE

## Pharmacological chaperones to cure genetic diseases - Q&A with G. Andreotti and M.V. Cubellis

September 4, 2013

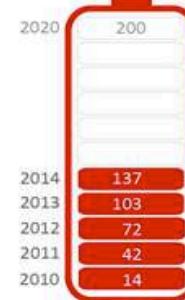
Dr Giuseppina Andreotti (scientific research council) and Dr Maria Vittoria Cubellis (coordinator), are working on the project *Pharmacological chaperones to cure genetic diseases: development of drugs and identification of new targets* (Telethon Foundation, Italy). In this interview, they will explain which are the problems they will tackle, how they will do so, and how it will help IRDiRC to reach its goals.

### Interview of Dr Giuseppina Andreotti and Dr Maria Vittoria Cubellis



Search...

### NEW THERAPIES



Objective 2020: 200 new therapies

Disclaimer: the numbers do not reflect IRDiRC initiatives only.

[More information](#)

### SPOTLIGHT ON IRDiRC



## RESEARCH ARTICLE

# Identification of an Allosteric Binding Site on Human Lysosomal Alpha-Galactosidase Opens the Way to New Pharmacological Chaperones for Fabry Disease



CrossMark  
click for updates

Valentina Citro<sup>1\*</sup>, Jorge Peña-García<sup>2\*</sup>, Helena den-Haan<sup>2</sup>, Horacio Pérez-Sánchez<sup>2\*</sup>, Rosita Del Prete<sup>1</sup>, Ludovica Liguori<sup>1,3</sup>, Chiara Cimmaruta<sup>1,3</sup>, Jan Lukas<sup>4</sup>, Maria Vittoria Cubellis<sup>1\*</sup>, Giuseppina Andreotti<sup>3</sup>

**1** Dipartimento di Biologia, Università Federico II, Napoli, 80126, Italy, **2** Bioinformatics and High Performance Computing Research Group (BIO-HPC), Computer Engineering Department, Universidad Católica San Antonio de Murcia (UCAM), Spain, **3** Istituto di Chimica Biomolecolare–CNR, Pozzuoli, 80078, Italy, **4** Albrecht-Kossel-Institute for Neuroregeneration, Medical University Rostock, Rostock, Germany

\* These authors contributed equally to this work.

\* [cubellis@unina.it](mailto:cubellis@unina.it) (MVC); [hperez@ucam.edu](mailto:hperez@ucam.edu) (HPS)

## OPEN ACCESS

**Citation:** Citro V, Peña-García J, den-Haan H, Pérez-Sánchez H, Del Prete R, Liguori L, et al. (2016) Identification of an Allosteric Binding Site on Human Lysosomal Alpha-Galactosidase Opens the Way to New Pharmacological Chaperones for Fabry Disease. PLoS ONE 11(10): e0165463. doi:10.1371/journal.pone.0165463

**Editor:** Stephan N. Witt, Louisiana State University Health Sciences Center, UNITED STATES

**Received:** May 10, 2016

**Accepted:** October 12, 2016

**Published:** October 27, 2016

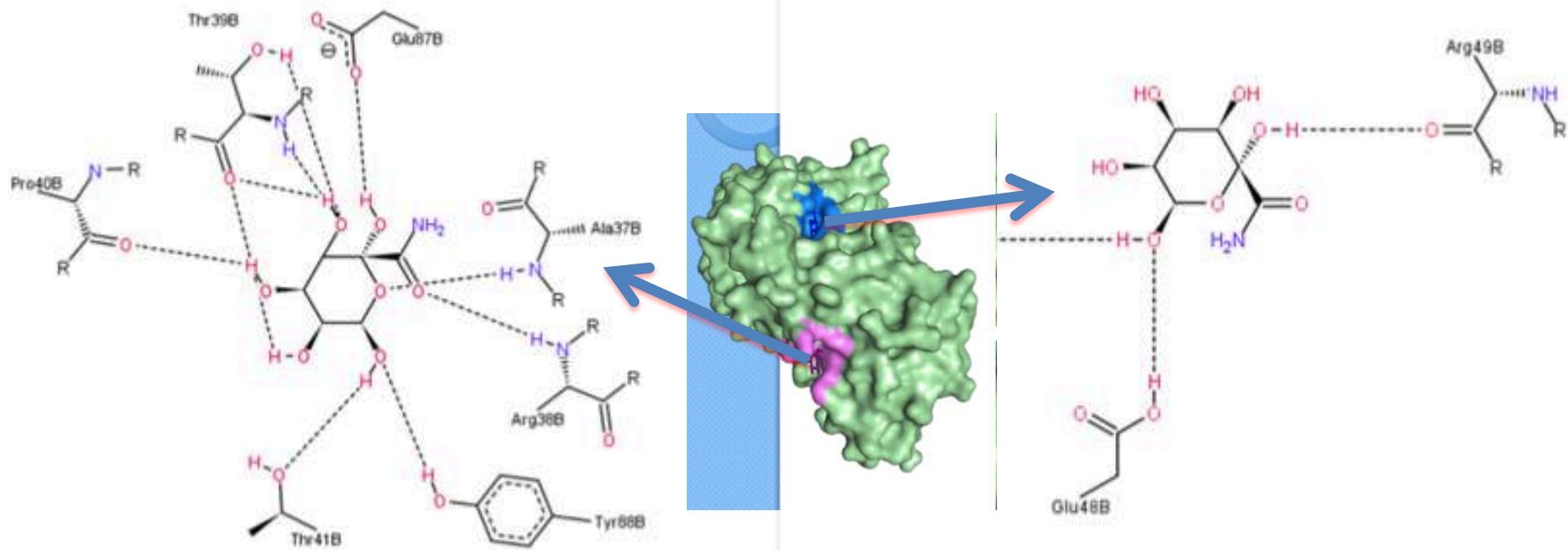
**Copyright:** © 2016 Citro et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which

## Abstract

Personalized therapies are required for Fabry disease due to its large phenotypic spectrum and numerous different genotypes. In principle, missense mutations that do not affect the active site could be rescued with pharmacological chaperones. At present pharmacological chaperones for Fabry disease bind the active site and couple a stabilizing effect, which is required, to an inhibitory effect, which is deleterious. By *in silico* docking we identified an allosteric hot-spot for ligand binding where a drug-like compound, 2,6-dithiopurine, binds preferentially. 2,6-dithiopurine stabilizes lysosomal alpha-galactosidase *in vitro* and rescues a mutant that is not responsive to a mono-therapy with previously described pharmacological chaperones, 1-deoxygalactonojirimycin and galactose in a cell based assay.

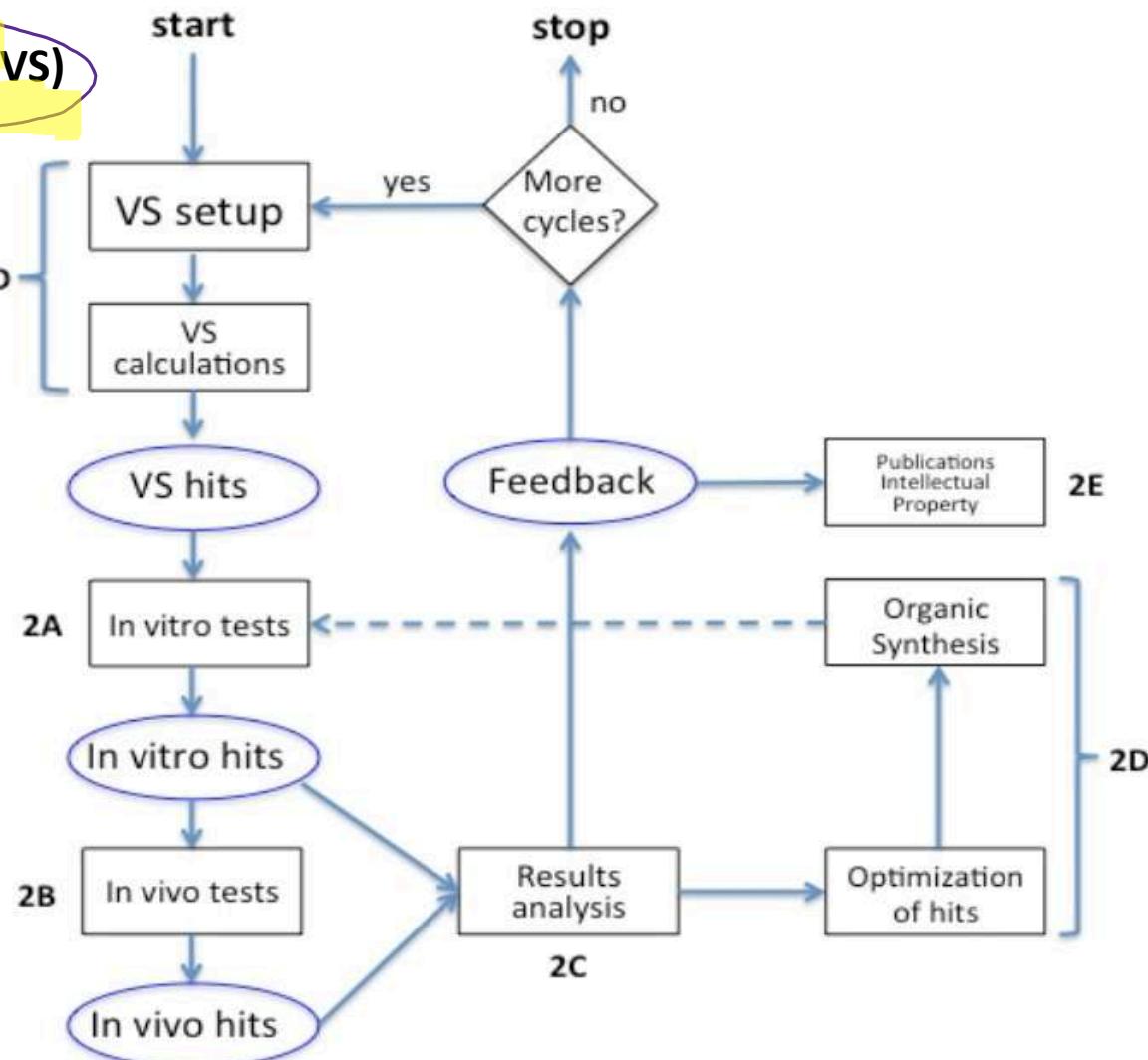
# COLLABORATION WITH UNIVERSITY OF NAPLES OUR PREDICTIONS FOR ALLOSTERIC SITE BINDERS

ZINC34800340



DEVELOPMENT OF THE  
BLIND DOCKING APPROACH

## VIRTUAL SCREENING (VS)

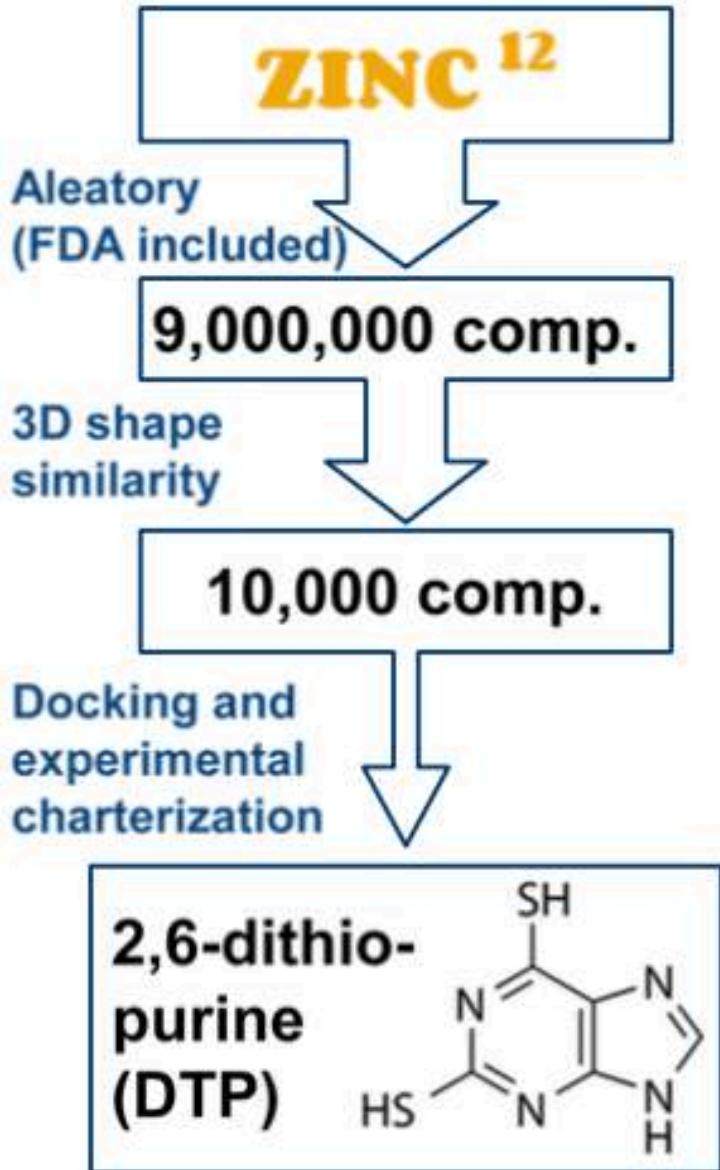


**Figure 4:** Depiction of the integrated computational-experimental strategy for bioactive compound discovery proposed for this project.

# “VIRTUAL” COMPOUND LIBRARIES

- DrugBank
    - FDA and experimental compounds (CS) → repurposing → quickest and cheapest gap to the market
    - Less than 10.000 CS
  - ChEMBL
    - Around 2.000.000 CS → more diversity than with DrugBank and perhaps more chances
    - “Academic CS” → still need “Hit to Lead” and in-vivo and clinical studies in most cases
    - Patenting issues
-  ZINC
- Around 40.000.000 CS → >> ChEMBL
  - No publications in most of the cases → patenting
  - Derived from commercial catalogs → uncertain origin and toxicity
  - Computationally expensive
- Virtual generated libraries
    - Using feedback or scaffolds from literature proposed by SHIRE
    - Generation of new virtual libraries after each cycle
  - Own PHARMA COMPANY libraries

# Methods and Materials



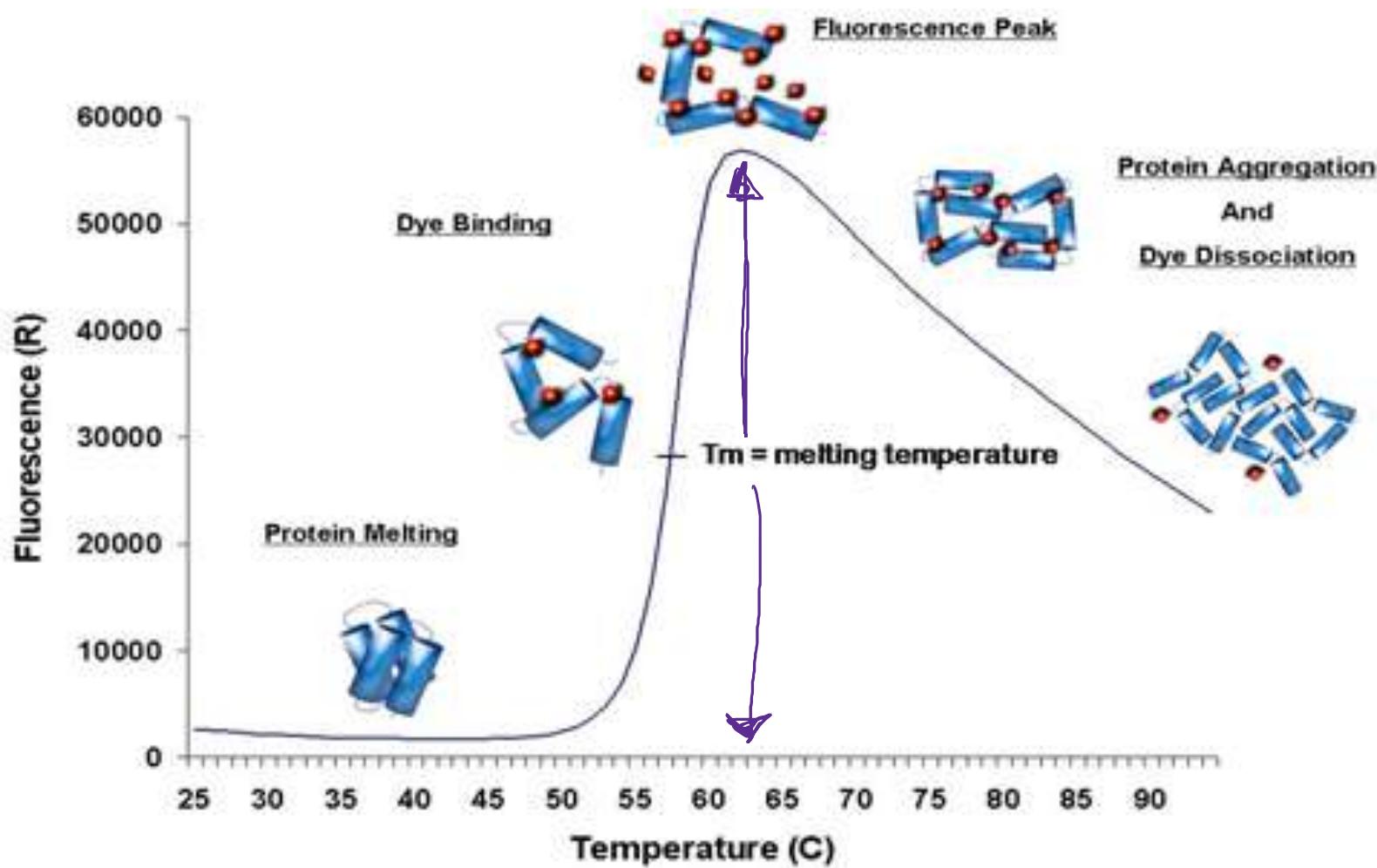
## Virtual Screening:

1. 3D shape similarity tool WEGA. Molecules scoring more than 0,8 were retained.
2. Docking tool Leadfinder. Sites for docking: substrate binding site and an allosteric binding site.

## Experimental:

1. Thermal and Urea-induced unfolding. Wt-AGAL (Fabrazyme) +DMSO+DTP+DGJ
2.  $\alpha$ -galactosidase (AGAL) activity measure in cell extracts: DMSO+DTP+DGJ+Galactose+Ambroxol.

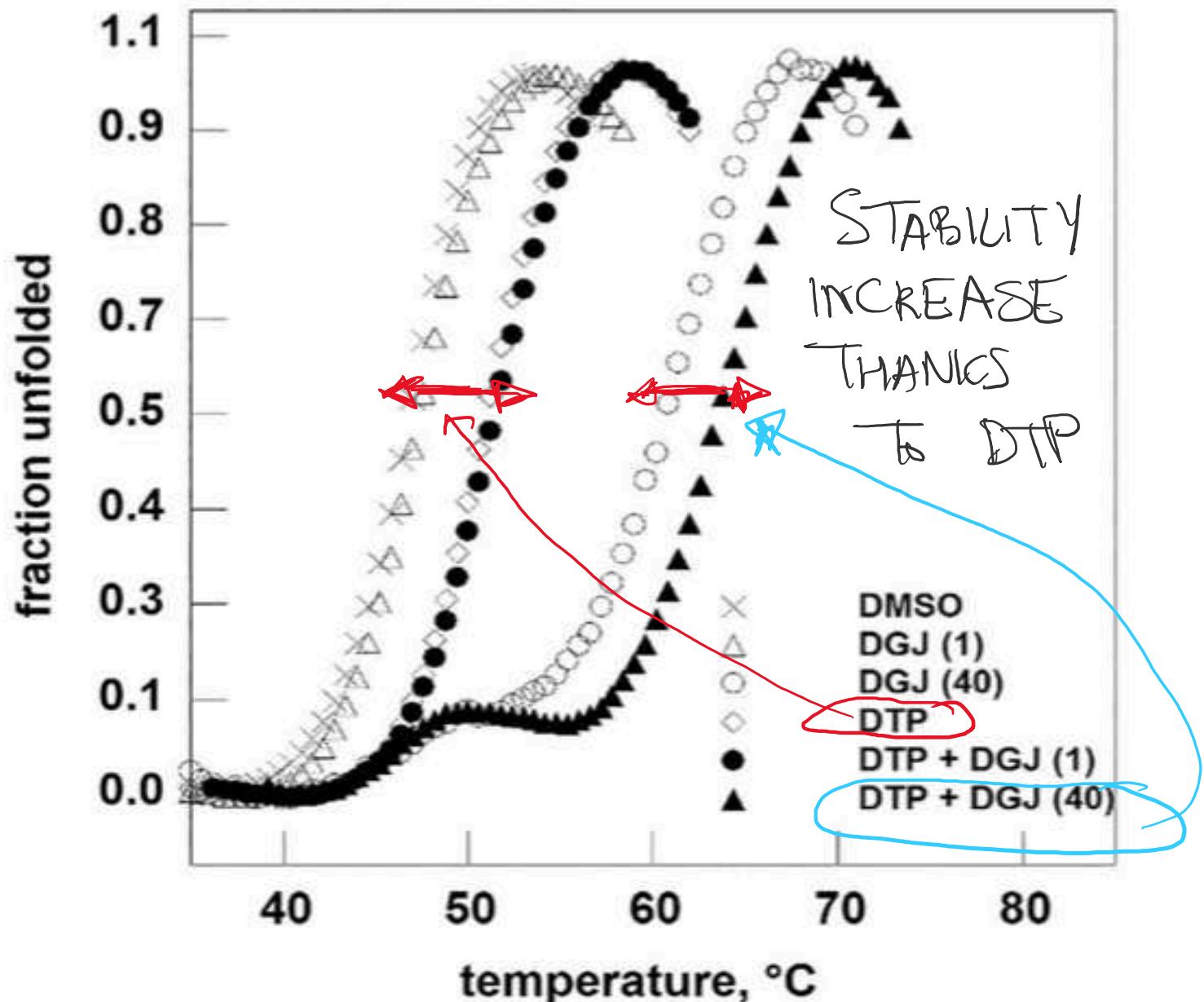
# THERMAL SHIFT ASSAY

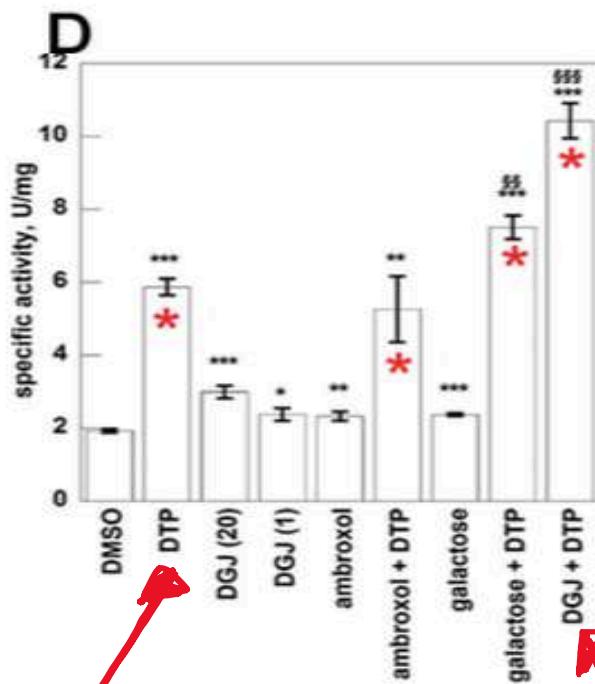
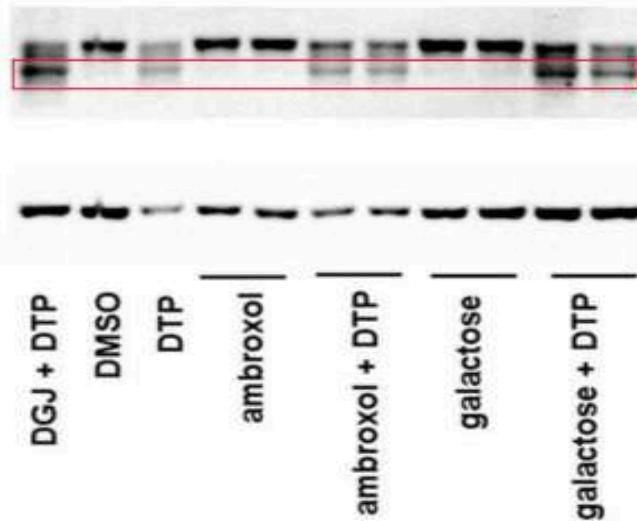
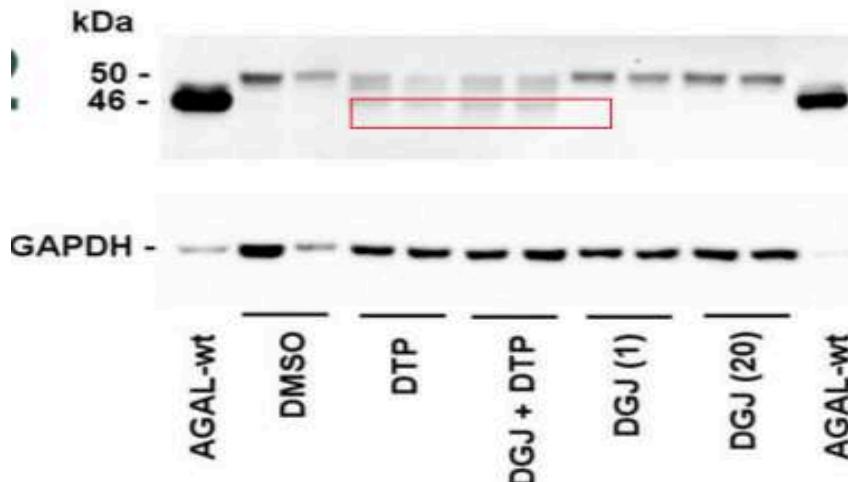


SYPRO-ORANGE DYE



PROTEIN





DTP rescues and enhances the activity of AGAL mutant A230T as a monotherapy or in synergy with other compounds as DGJ.

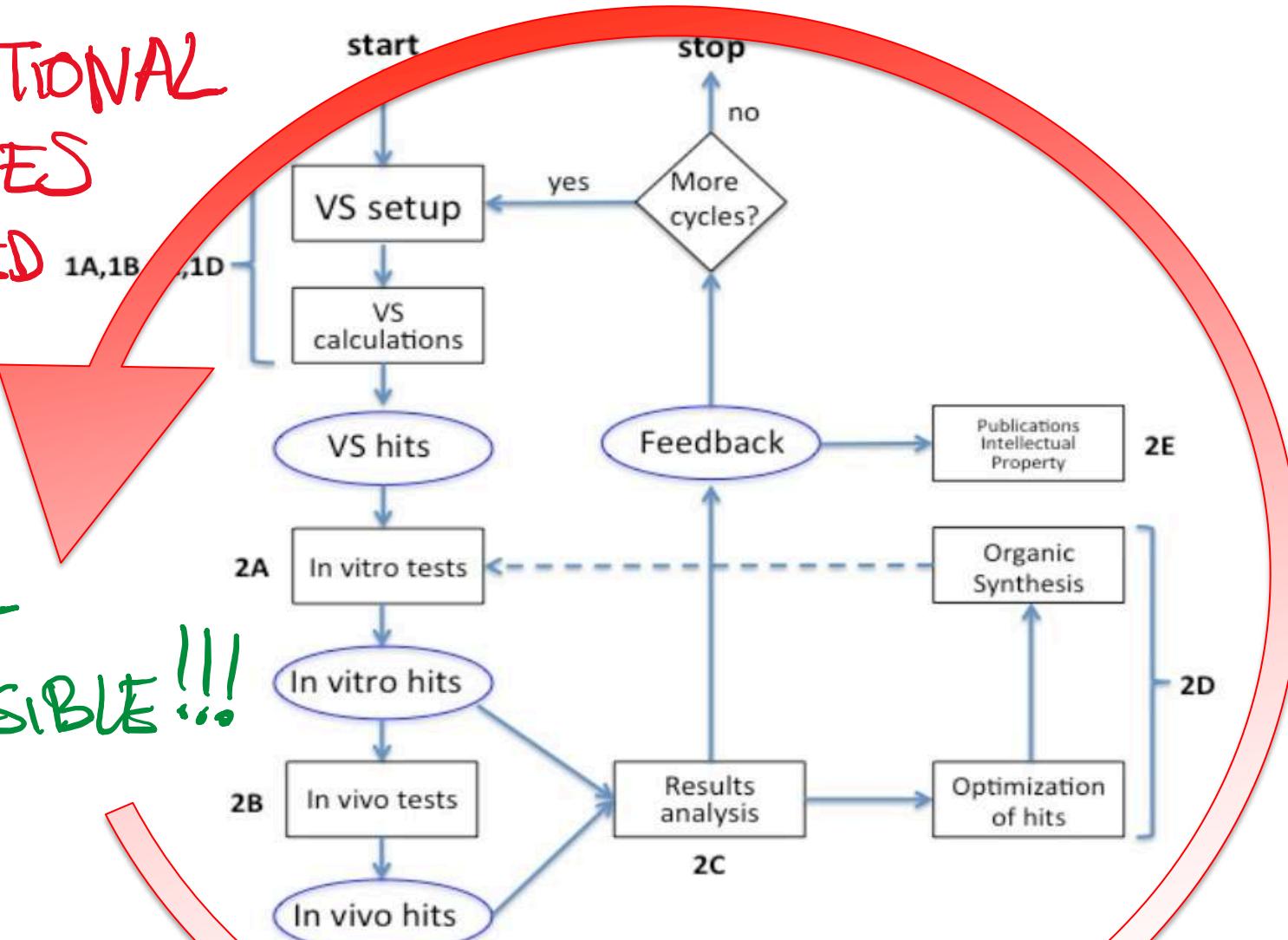
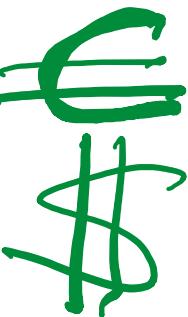
DTP does not interfere with enzymatic activity

# MAIN RESULTS AND CONCLUSIONS

- Identification of a compound (DTP) which has affinity for AGAL at allosteric site (few existing)
  - Predicted region: A37, Y38, T39, P40, T41, M42
- DTP is a promising molecule since:
  - it is currently used as chemopreventive agent
  - it is actively transported into mammalian cells
  - it is safe
- DTP promotes processing of some mutants non responsive to DGJ as monotherapy or in synergy with other compounds
- Main result obtained is the molecule AND the validation of our methodology in the context of molecular allosteric chaperones

**ADDITIONAL CYCLES REQUIRED**

**NOT POSSIBLE !!!**



**Figure 4:** Depiction of the integrated computational-experimental strategy for bioactive compound discovery proposed for this project.

# NEXT STEPS !

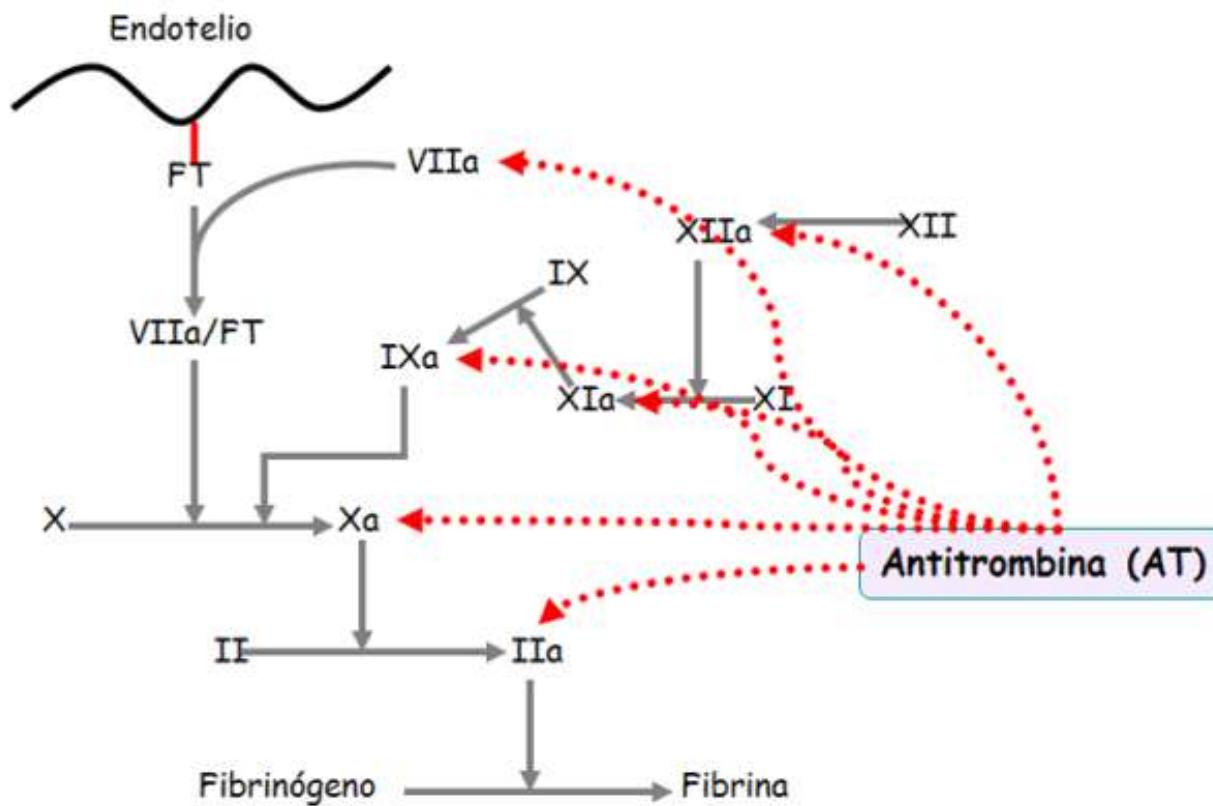
- Crystallographic structure of DTP-AGAL complex
- Chemical optimization of DTP
- Collaborations with other research groups **and companies** sought
  - Fabry and other lysosomal diseases
- Joint application to grant calls (H2020, NIH, NSF, etc)
- Exploitation of obtained experimental feedback for reprocessing of compound databases

# BIO-HPC research group Drug Discovery pipeline

CONTEXT	COMPOUND TYPE	DEVELOPMENT STAGE				IP STATUS
		IN SILICO	IN VITRO	IN VIVO	CLINICAL STUDIES	
Zika virus inhibitor	FDA				2020	Patent licensed
Colorectal cancer	FDA 1				Requested	Patent filed
	FDA 2				Requested	Patent filling in process
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Xylella fastidiosa	Natural compounds				In process	



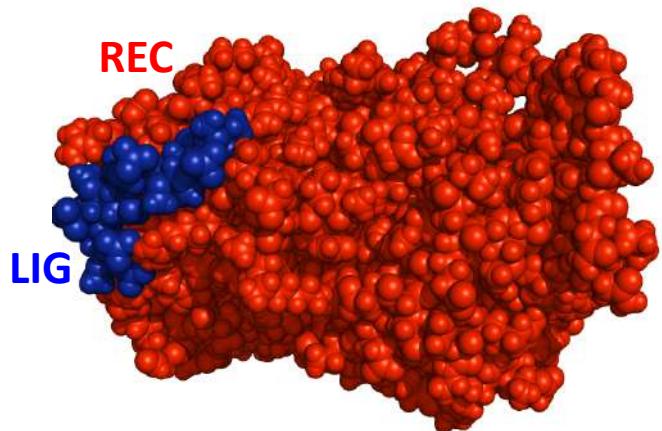
# APPLICATION PROBLEM; ANTICOAGULANT THERAPY



Principal  
anticoagulante  
endógeno

UCAM

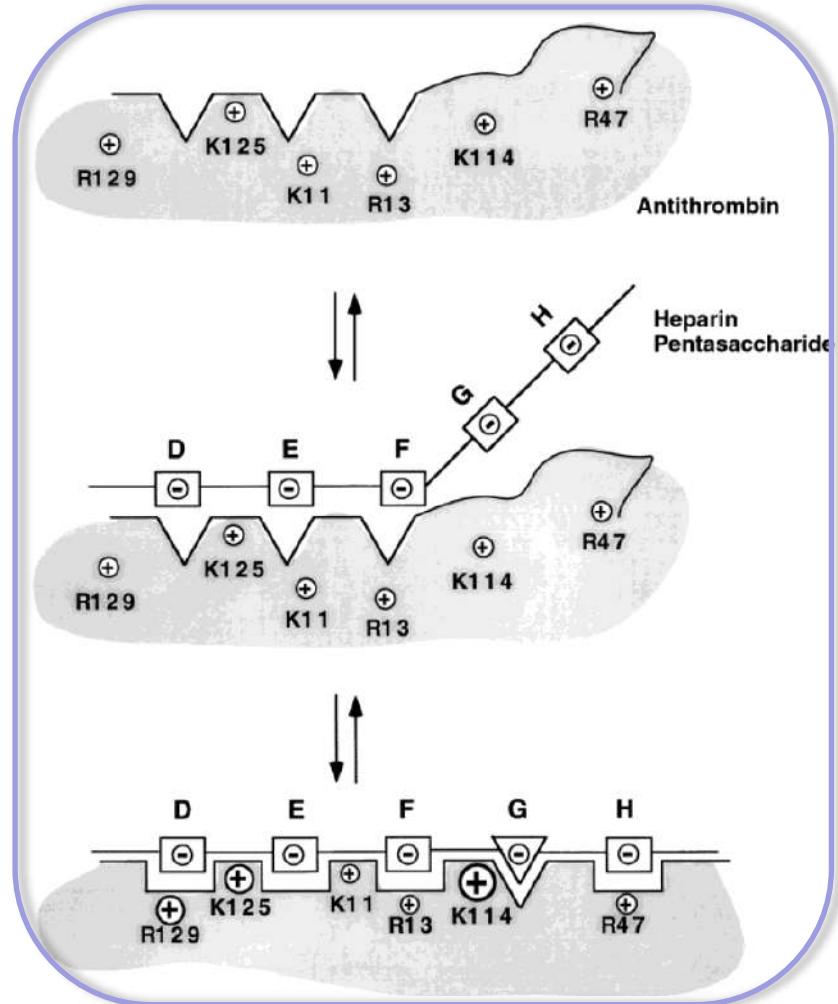
# ANTITHROMBIN AND HEPARIN



Deficiency of AT type II in  
heparin binding site



Heparin is useless here



# EXTENSIVE SEARCH FOR HEPARIN ALTERNATIVES



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Bioorganic & Medicinal Chemistry 12 (2004) 633–640

Bioorganic &  
Medicinal  
Chemistry

## Hydropathic interaction analyses of small organic activators binding to antithrombin

Gunnar T. Gunnarsson and Umesh R. Desai\*

Department of Medicinal Chemistry, Virginia Commonwealth University, 410 N. 12th Street, PO Box 980540, Richmond, VA 23298, USA

Received 8 September 2003; accepted 11 October 2003

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 679–683

## Exploring New Non-sugar Sulfated Molecules as Activators of Antithrombin

Gunnar T. Gunnarsson and Umesh R. Desai\*

Department of Medicinal Chemistry, Virginia Commonwealth University,  
410 N. 12th Street, PO Box 980540, Richmond, VA 23298, USA

Received 3 September 2002; accepted 14 November 2002



J Med Chem. 2002 Sep 26;45(20):4460-70.

## Interaction of designed sulfated flavanoids with antithrombin: lessons on the design of organic activators.

Gunnarsson GT, Desai UR.

Department of Medicinal Chemistry, Virginia Commonwealth University, 410 North 12th Street, P.O. Box 980540, Richmond, Virginia 23298, USA.



J. Biol. Chem. 2009 284: 20897–20908.

## Interaction of antithrombin with sulfated, low molecular weight lignins: opportunities for potent, selective modulation of antithrombin function.

Henry BL, Connell J, Liang A, Krishnasamy C, Desai UR.

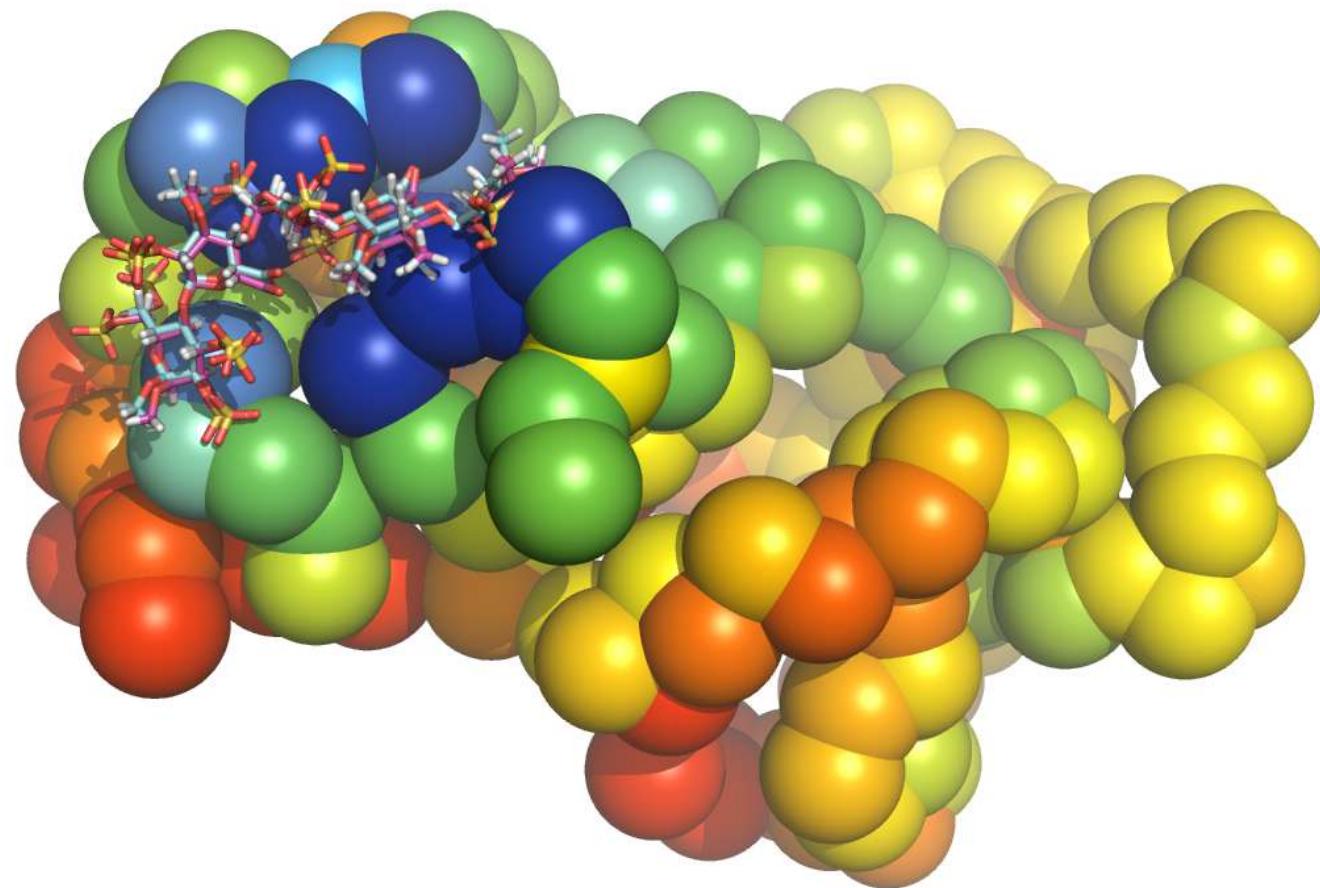
Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, Virginia 23298, USA.

**UCAM**

Reunión Nacional AEHH  
XXVI Congreso Nacional SETH

Las Palmas de Gran Canaria • 28-30 Octubre 2010

# PROTEIN SURFACE BLIND FLEXIBLE DOCKING: Antithrombin and heparin



- Ligands with up to 200 at., 20 rotatable bonds, receptor flexibility (sidechains, backbone)
- Collaboration with Faculty of Medicine, University of Murcia: Experimental determination of the activity of predicted compounds

# “VIRTUAL” COMPOUND LIBRARIES

- DrugBank
    - FDA and experimental compounds (CS) → repurposing → quickest and cheapest gap to the market
    - Less than 10.000 CS
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    - Using feedback or scaffolds from literature proposed by SHIRE
    - Generation of new virtual libraries after each cycle
  - Own PHARMA COMPANY libraries

# IN-SILICO SCREENING SELECTED COMPOUNDS



We screened 13 millions of ligands and selected 10 for in vitro testing

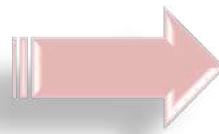
Collaboration with Centro de Hemodonación  
Faculty of Medicine, University of Murcia

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LII Reunión Nacional AEHH  
XXVI Congreso Nacional SETH

Las Palmas de Gran Canaria • 28-30 Octubre 2010

# IN-SILICO SCREENING SELECTED COMPOUNDS



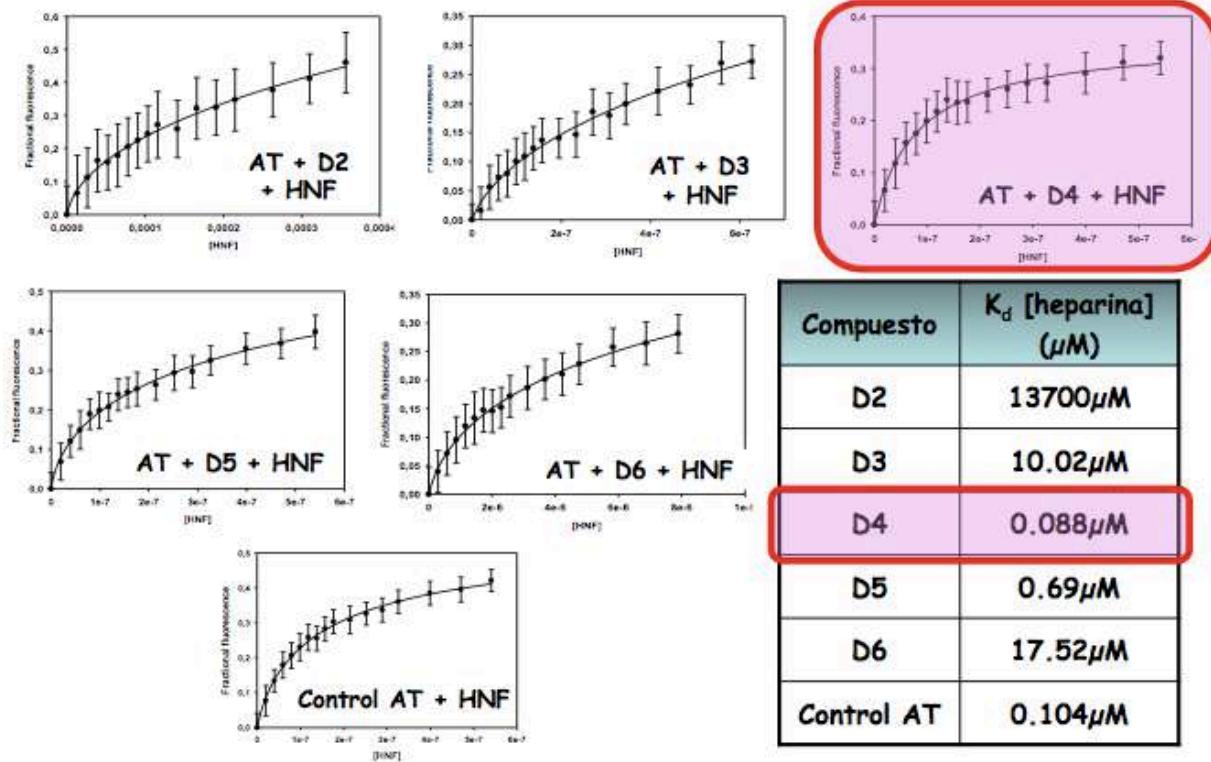
We screened 13 millions of ligands and selected 10 for in vitro testing

Nombre del compuesto	Resultado	Scoring Function
D1	Autohidrólisis	-728
D2	Seleccionado	-966
D3	Seleccionado	-1062
D4	Seleccionado	-2300
D5	Seleccionado	-751
D6	Seleccionado	-993
D7	Insoluble en agua	-864
D8	Insoluble en agua	-1005
Heparina	---	-1100

Collaboration with Centro de Hemodonación  
Faculty of Medicine, University of Murcia

# Discovery of a novel scaffold

Experimental results for the top 5 compounds resulting from the in-silico screening, measuring changes of intrinsic fluorescence [4]. Isothermal titration calorimetry, in vivo citrullination of Antithrombin, electrophoretic studies and studies in plasma samples give similar results and confirm the predictive capability of the methodology [7].



## DISCOVERY OF THE COMPOUND PATENTED (EU)

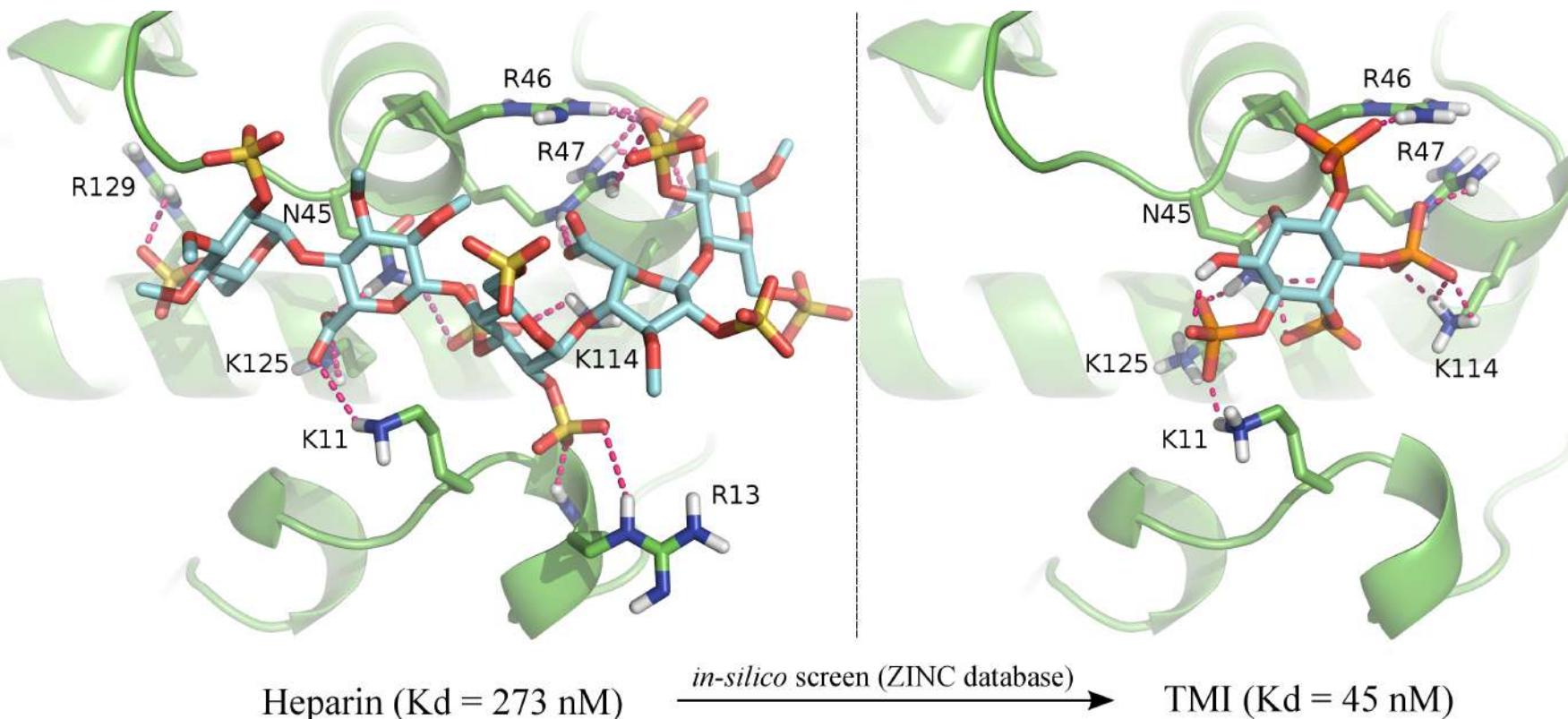
Pérez-Sánchez, I. Meliciani, W. Wenzel, I. Martínez-Martínez, J. Navarro-Fernández, J. Corral, V. Vicente-García, "A Molecular Scaffold to Modulate Thrombin/Antithrombin Activity by Heparin Binding". European patent application.

## PUBLICATION

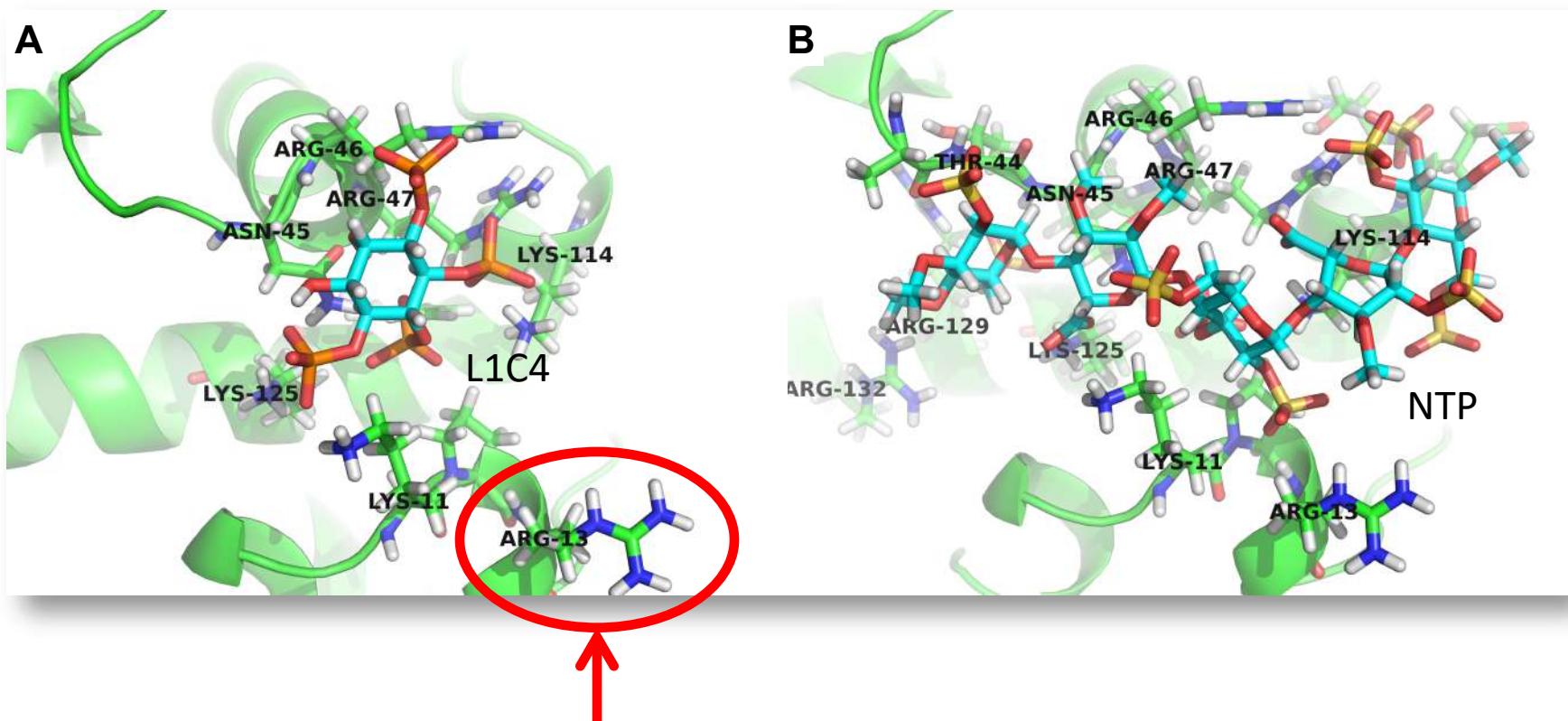
J. Navarro-Fernández, H. Pérez-Sánchez, I. Martínez-Martínez, I. Meliciani, J.A. Guerrero, V. Vicente, J. Corral, W. Wenzel, (2011) "Discovery of a compound that binds with nanomolar affinity to antithrombin by structural docking: experimental validation and functional consequences", (submitted).

## In Silico Discovery of a Compound with Nanomolar Affinity to Antithrombin Causing Partial Activation and Increased Heparin Affinity

J. Navarro-Fernández,<sup>†,||</sup> H. Pérez-Sánchez,<sup>‡,§,||</sup> I. Martínez-Martínez,<sup>†</sup> I. Meliciani,<sup>‡</sup> J. A. Guerrero,<sup>†</sup>  
V. Vicente,<sup>†</sup> J. Corral,<sup>\*,†</sup> and W. Wenzel<sup>\*,‡</sup>



# Key residues in the active site of antithrombin when complex with TMI and Heparin

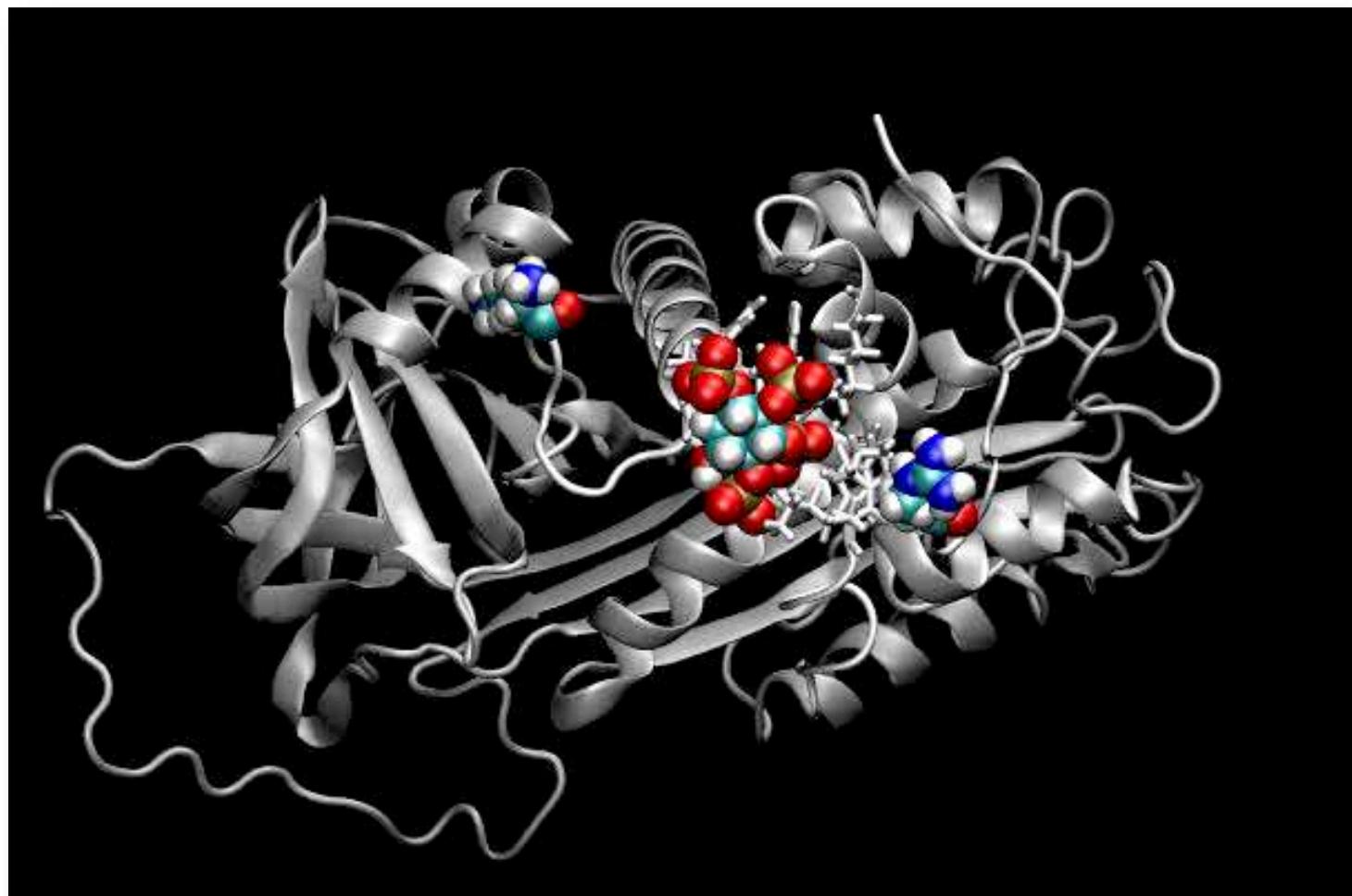


No contacts between ARG13 and L1C4  
in the initial structure.

Movie of Heparin MD trajectory shows no major changes in interactions between Ligand and protein



Movie of TMI trajectory reveals Arg13 and Lys39 form hydrogen bonds with ligand after a few ns.

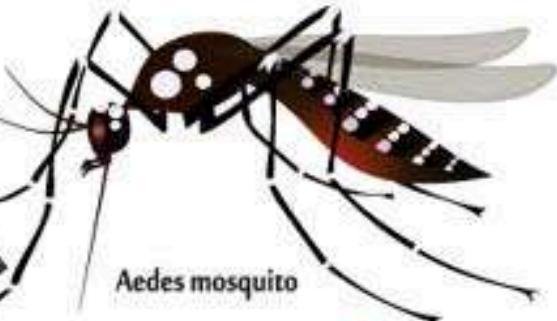


# BIO-HPC research group Drug Discovery pipeline

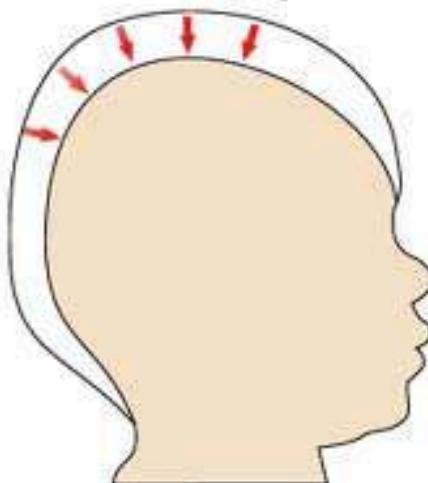


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Lipase inhibitor	FDA					
Fabry disease	Chemical safe compound					Paper published
Anticoagulants	Human endogenous compound				Requested	Patented and published
Inflamasoma	FDA				In process	
Antibacterials in odontology	FDA				In process	
Xylella fastidiosa	Natural compounds				In process	

# ZIKA VIRUS



Microcephaly



## Microcephaly

Symptoms include  
below-average  
head size

Often caused by  
failure of brain to  
grow at normal rate

Head circumference  
measuring less than  
31-32cm

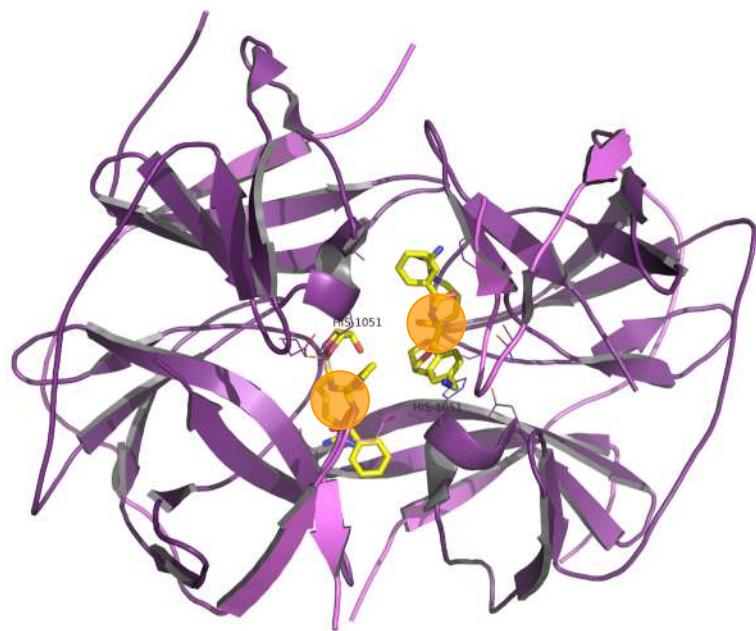
# “VIRTUAL” COMPOUND LIBRARIES

## DrugBank

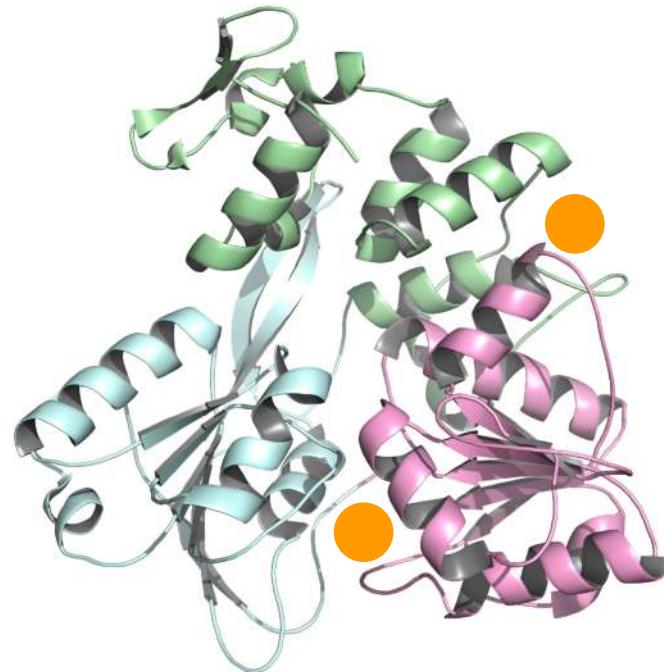
- FDA and experimental compounds (CS) → repurposing → quickest and cheapest gap to the market
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  - No publications in most of the cases → patenting
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  - Computationally expensive
- Virtual generated libraries
  - Using feedback or scaffolds from literature proposed by SHIRE
  - Generation of new virtual libraries after each cycle
- Own PHARMA COMPANY libraries

# Chosen targets: Zika disease

Protease NS2B-NS3  
PDB code 5LC0



NS3 Helicase  
PDB code 5JMT

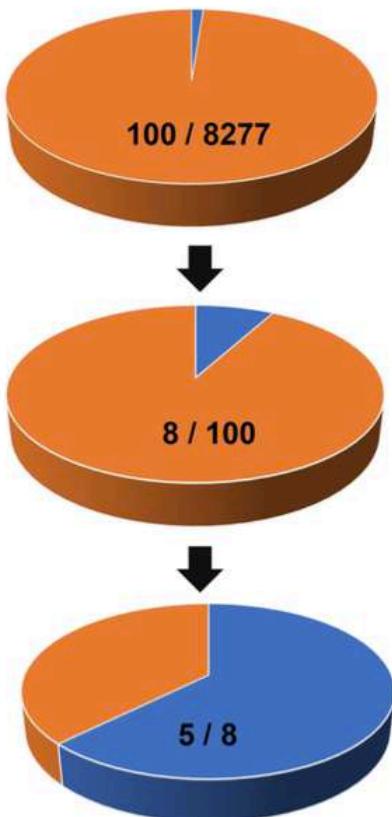


Involved in viral replication

*In silico* structure-based virtual screening:  
identification of top 100 primary hit compounds

Primary screening:  
selection of 8 clinically approved drugs belonging to different drug classes for further validation studies

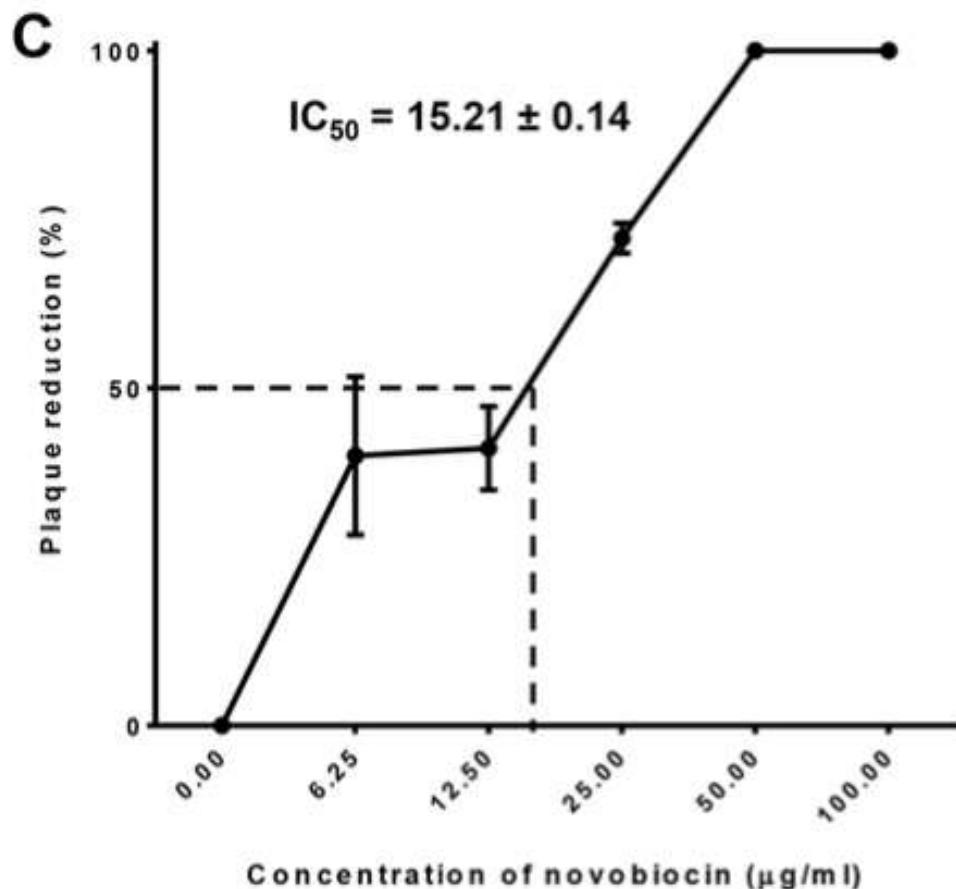
Validation of protease inhibition: ZIKV-NS2B-NS3 fluorescence-based protease inhibition assay



## DRUG REPURPOSING APPROACH

### VIRTUAL SCREENING (VS) CALCULATIONS

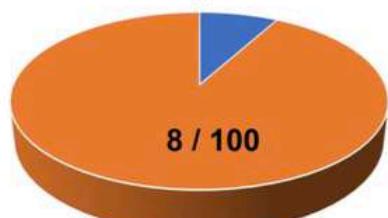
- Carried out with ShuttleMol
- HPC scripts suite focused for VS
- Advanced VS results analysis
- Consensus scoring



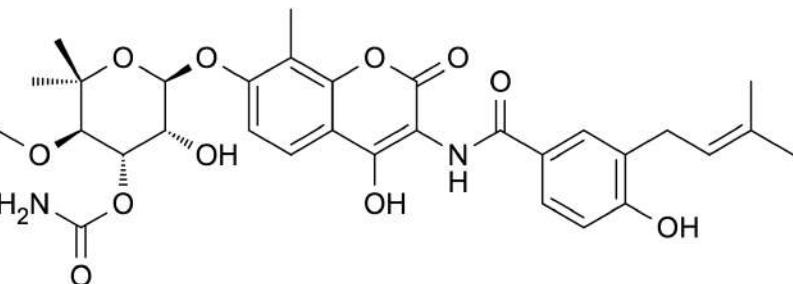
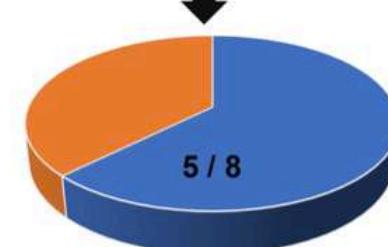
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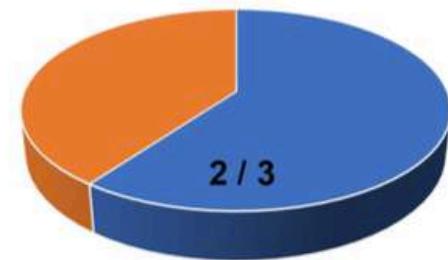


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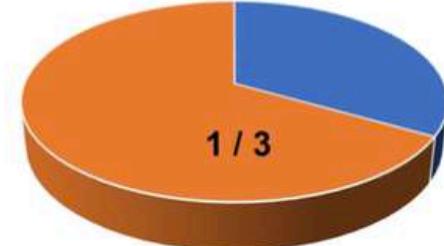


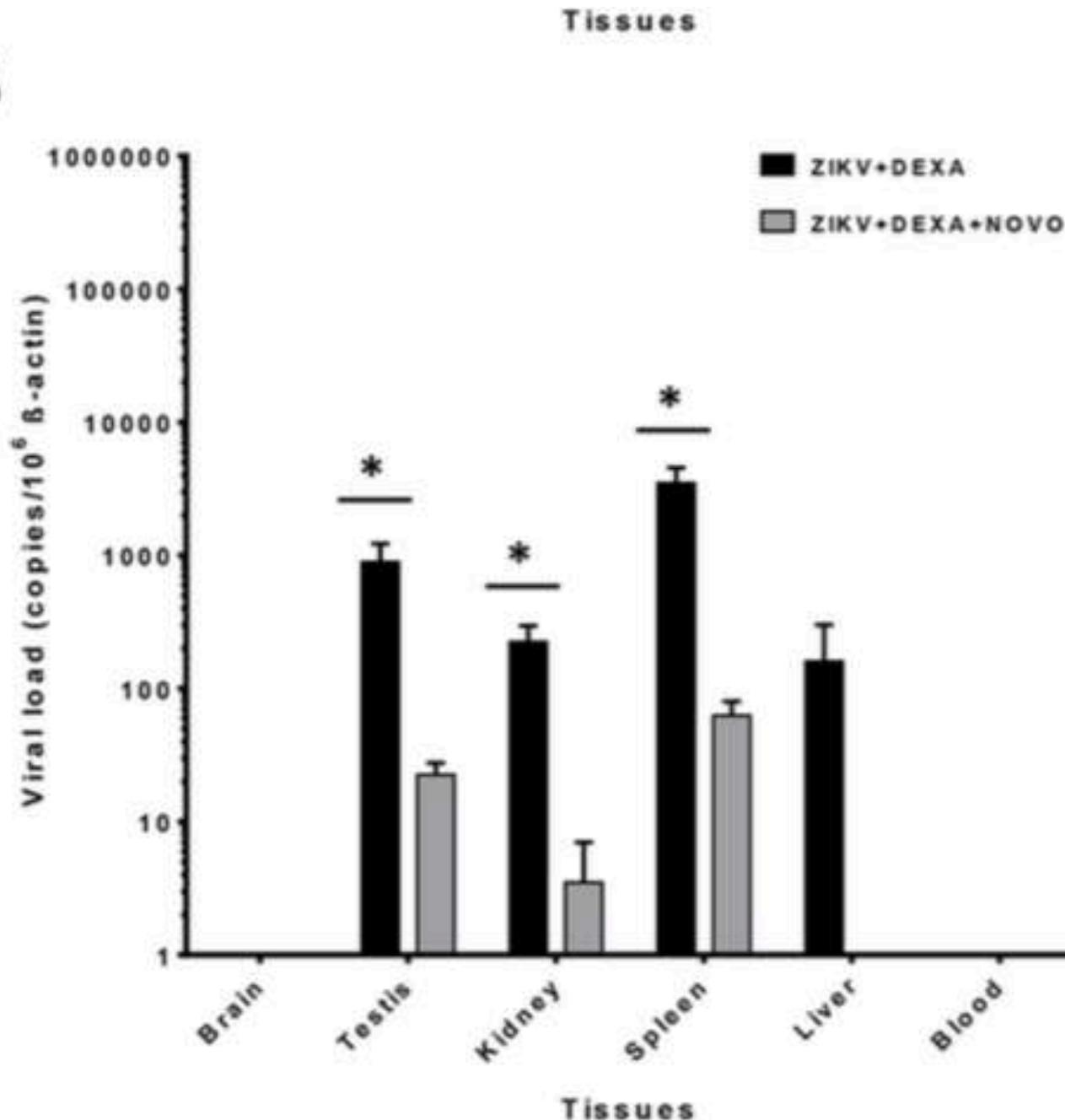
NOVOBIOCIN

Validation of *in vitro* anti-ZIKV activity: further selection of 3 validated ZIKV-NS2B-NS3 protease inhibitors that have high potentials for clinical use



Validation of *in vivo* anti-ZIKV activity: selection of novobiocin for evaluation in mouse model based on its optimal pharmacological properties



**B**



## Structure-based discovery of clinically approved drugs as Zika virus NS2B-NS3 protease inhibitors that potently inhibit Zika virus infection *in vitro* and *in vivo*



Shuofeng Yuan <sup>a, 1</sup>, Jasper Fuk-Woo Chan <sup>a, b, c, d, \*\*, 1, 2</sup>, Helena den-Haan <sup>e, f, 1</sup>,  
 Kenn Ka-Heng Chik <sup>a</sup>, Anna Jinxia Zhang <sup>a</sup>, Chris Chung-Sing Chan <sup>a</sup>,  
 Vincent Kwok-Man Poon <sup>a</sup>, Cyril Chik-Yan Yip <sup>a</sup>, Winger Wing-Nga Mak <sup>a</sup>, Zheng Zhu <sup>a</sup>,  
 Zijiao Zou <sup>a</sup>, Kah-Meng Tee <sup>a</sup>, Jian-Piao Cai <sup>a</sup>, Kwok-Hung Chan <sup>a</sup>, Jorge de la Peña <sup>e</sup>,  
 Horacio Pérez-Sánchez <sup>e, \*\*\*, 2</sup>, José Pedro Cerón-Carrasco <sup>e, \*\*\*\*, 2</sup>,  
 Kwok-Yung Yuen <sup>a, b, c, d, g, \*, 2</sup>

<sup>a</sup> Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region

<sup>b</sup> State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region

<sup>c</sup> Research Centre of Infection and Immunology, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region

<sup>d</sup> Carol Yu Centre for Infection, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region

<sup>e</sup> Bioinformatics and High Performance Computing Research Group (BIO-HPC), Computer Engineering Department, Universidad Católica San Antonio de Murcia (UCAM), Spain

<sup>f</sup> Villapharma Research S.L., Parque Tecnológico de Fuente Álamo, Ctra. El Estrecho-Lobosillo, Km. 2.5, Av. Azul, Fuente Álamo de Murcia, Murcia, Spain

<sup>g</sup> The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region

- **18 authors !**
- **PCT PATENT FILED (JUNE 2017)**
- **PATENT LICENSED TO USA PHARMA COMPANY (JAN 2019) → CLINICAL TRIALS (2020)**
- **INTERNATIONAL CONSORTIUM ESTABLISHED (SPAIN, GERMANY, BRAZIL, USA)**

# BIO-HPC research group Drug Discovery pipeline

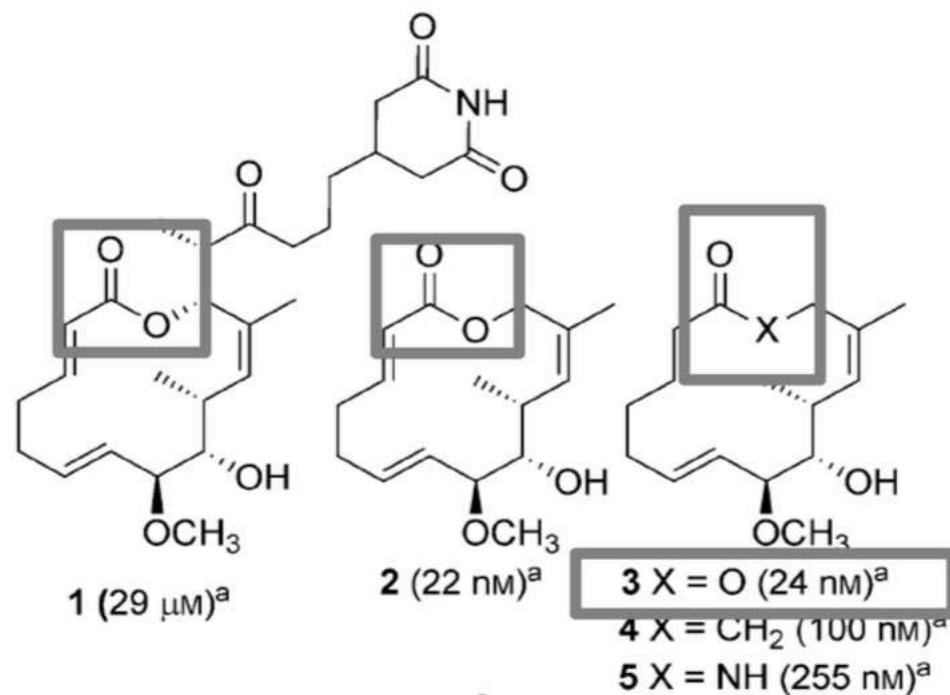


CONTEXT	COMPOUND TYPE	DEVELOPMENT STAGE				IP STATUS
		IN SILICO	IN VITRO	IN VIVO	CLINICAL STUDIES	
Zika virus inhibitor	FDA				2020	Patent licensed
Colorectal cancer	FDA 1				Requested	Patent filed
	FDA 2				Requested	Patent filling in process
	Merck compound				Requested	Patent filling in process
Anti-aging	Natural product				Requested	Patent filling in process
	FDA(s)				In process	
Gastric cancer	FDA x 2				In process	
Anti bacterial (MraY)	FDA				In process	
Weight loss - nicotinic receptor modulator	FDA				In process	
Lipase inhibitor	FDA					
Fabry disease	Chemical safe compound					Paper published
Anticoagulants	Human endogenous compound				Requested	Patented and published
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## LETTERS

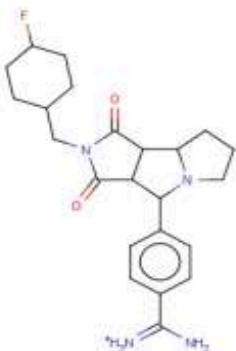
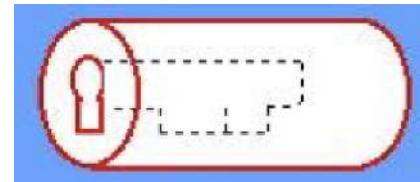
# Migrastatin analogues target fascin to block tumour metastasis

Lin Chen<sup>1\*</sup>, Shengyu Yang<sup>1\*</sup>, Jean Jakopic<sup>2</sup>, J. Jillian Zhang<sup>1</sup> & Xin-Yun Huang<sup>1</sup>





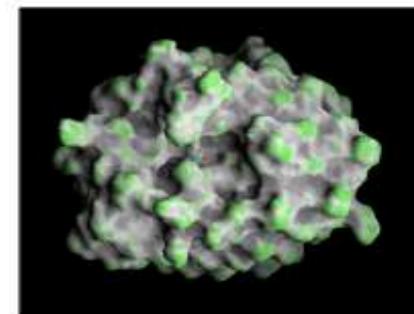
# Virtual Screening



3D Structure of Target

Unknown

Known



Ligand-Based  
Methods

Actives known

Actives **and** inactives known

Structure-Based  
Methods

Similarity  
searching

Pharmacophore  
mapping

Machine learning  
methods

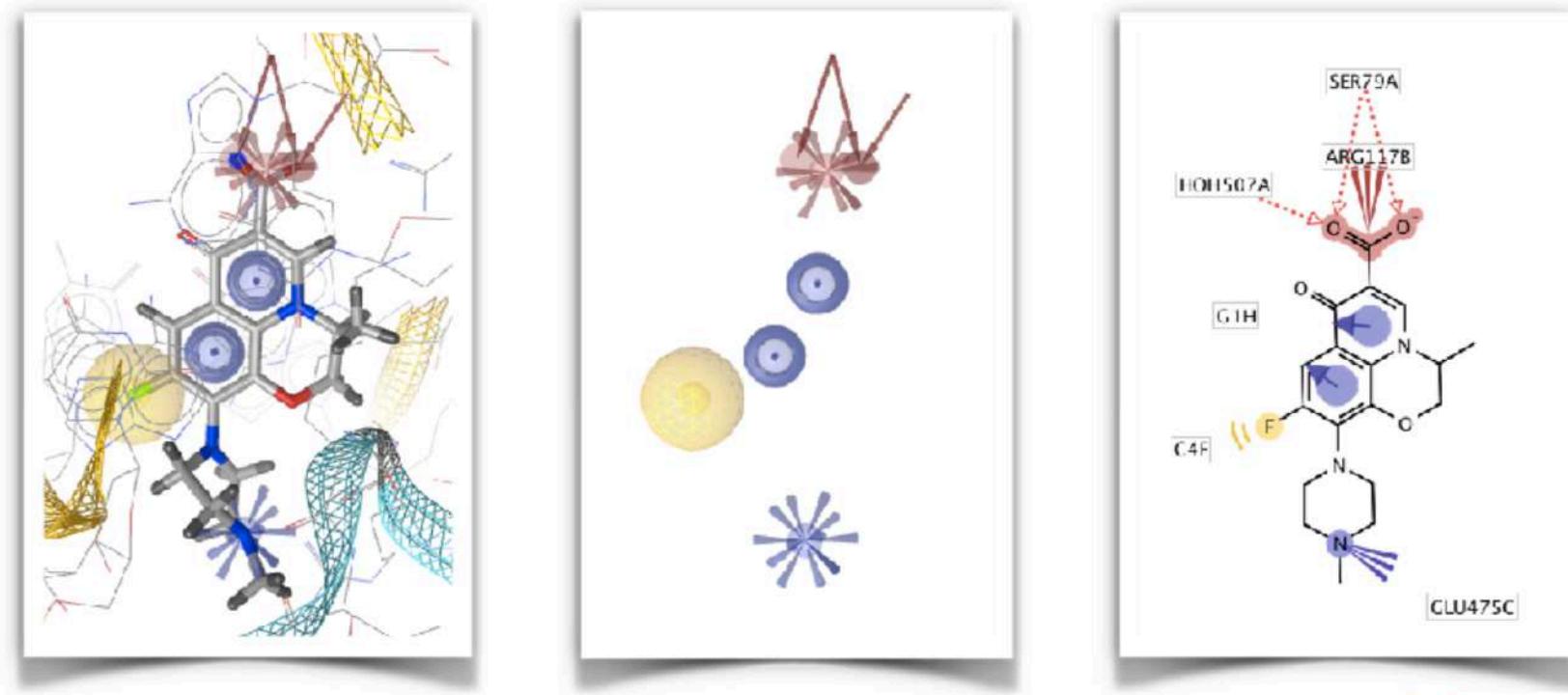
Protein Ligand  
Docking



# Feature-based Pharmacophores

Totality of universal chemical features that represent a defined binding mode of a ligand to a bio-molecular target

Features: Electrostatic interactions, H-bonding, aromatic interactions, hydrophobic regions, coordination to metal ions ...



# “VIRTUAL” COMPOUND LIBRARIES

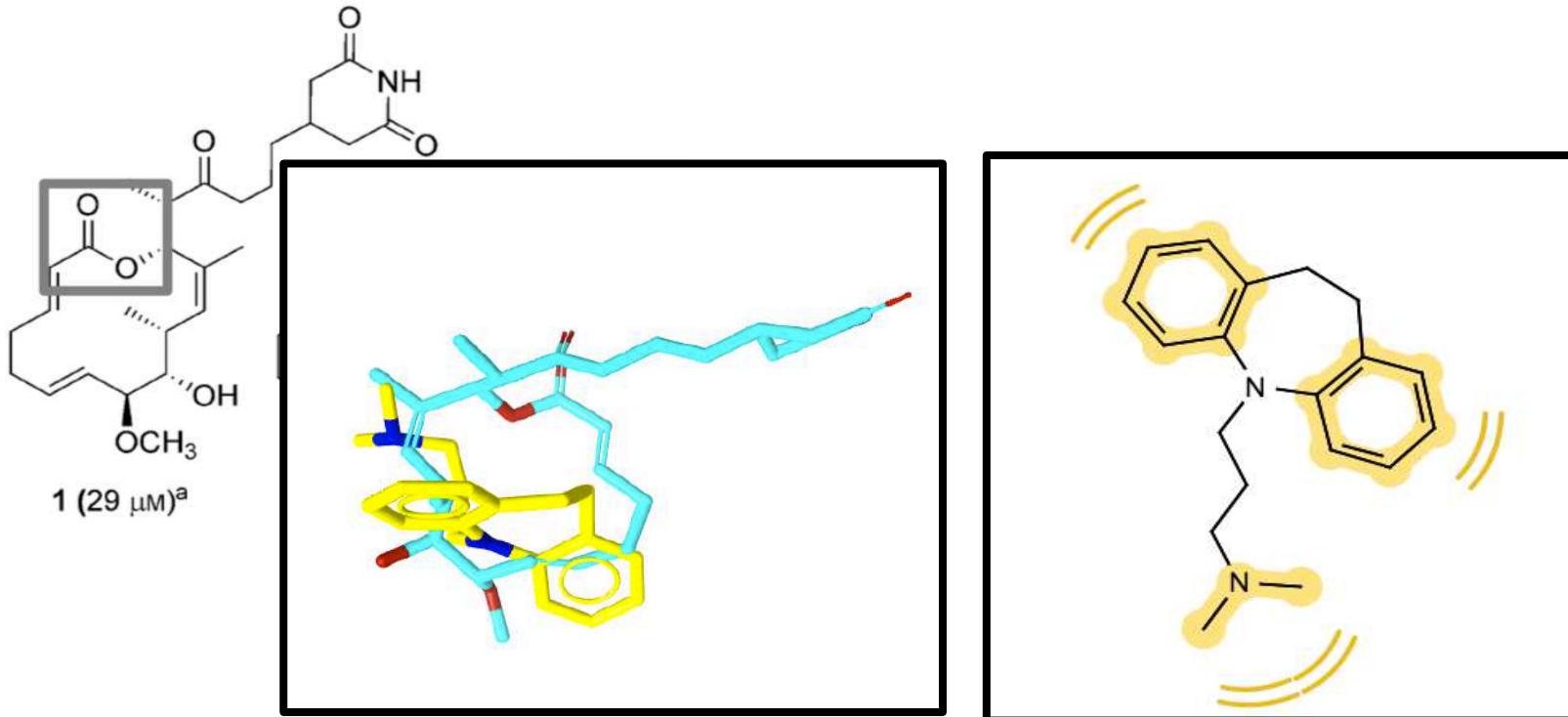
## DrugBank

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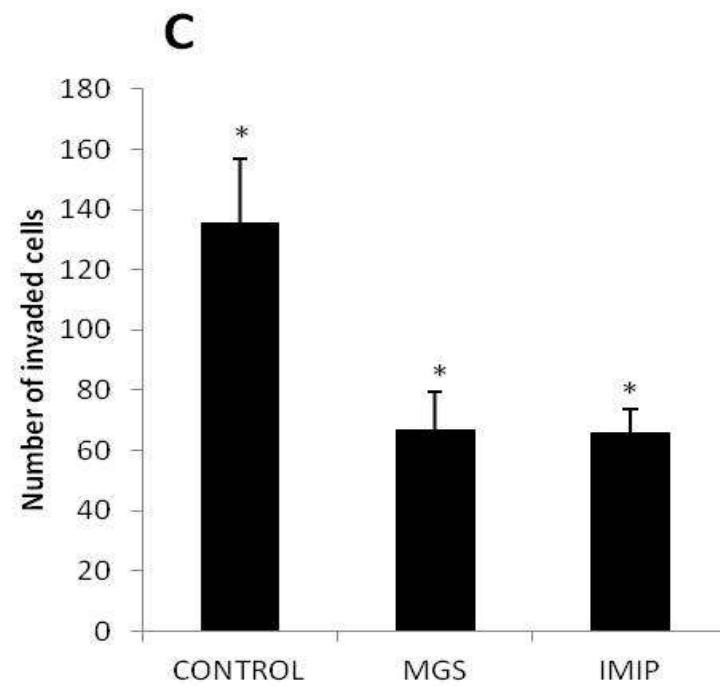
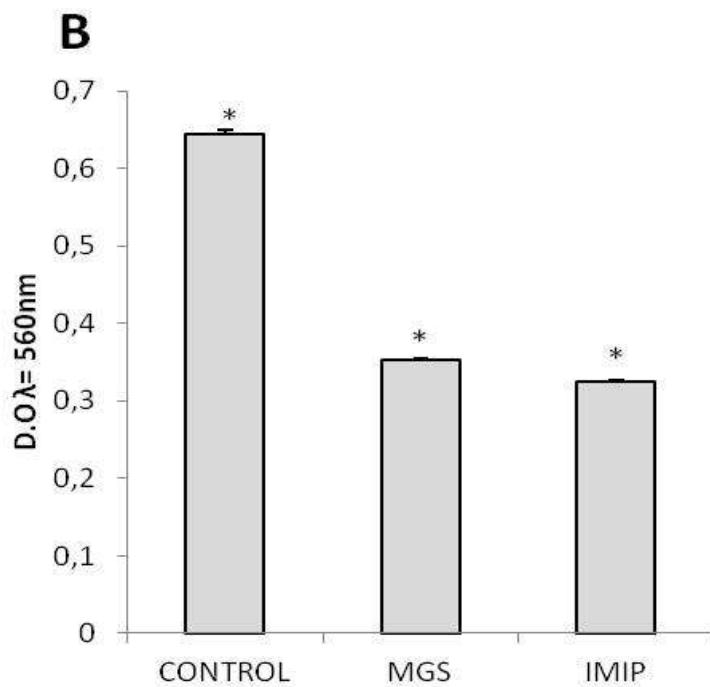
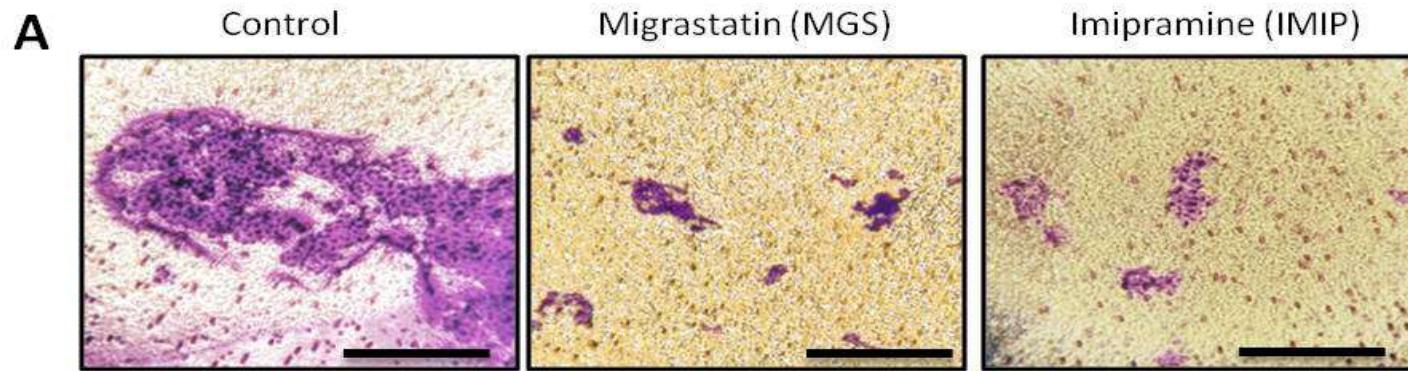
# IN COLORECTAL CANCER

## Field of the invention

The present invention relates to the field of medicinal products, particularly to  
therapeutic products against cancer.

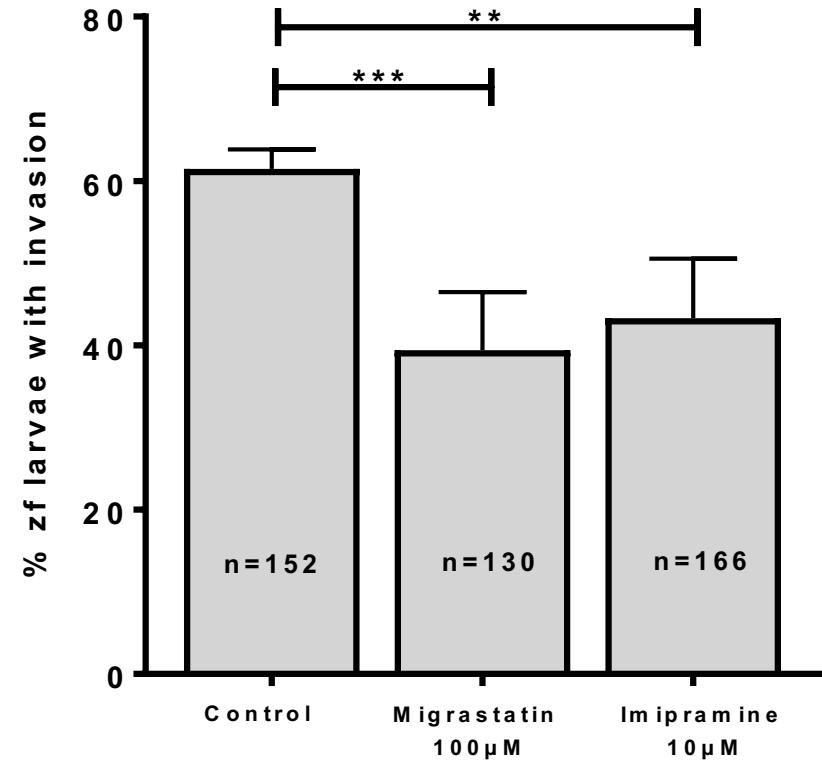
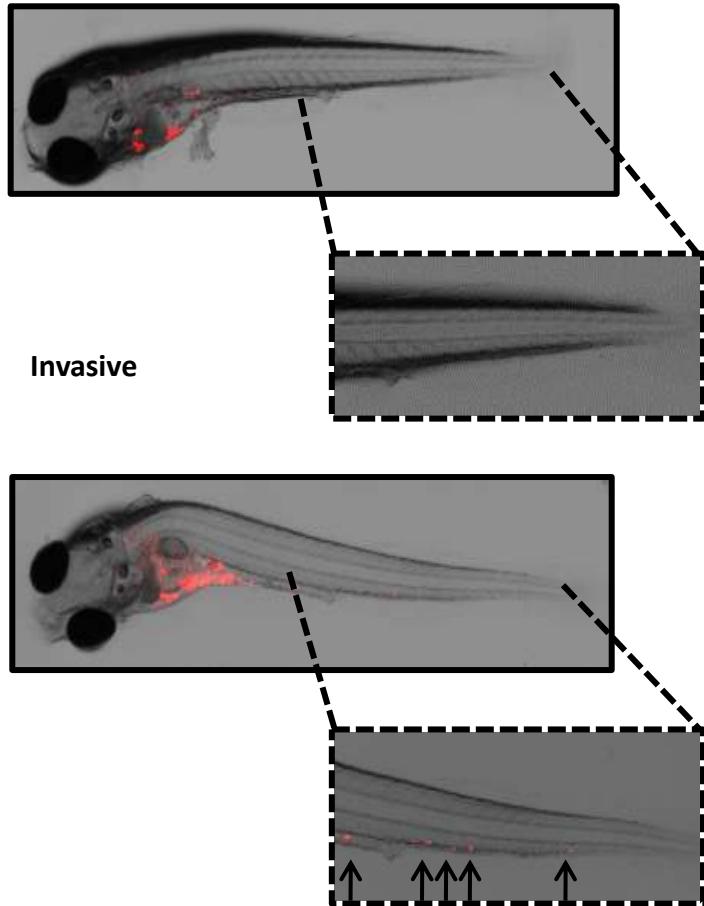


PHARMACOPHORE LIGAND BASED VIRTUAL SCREENING



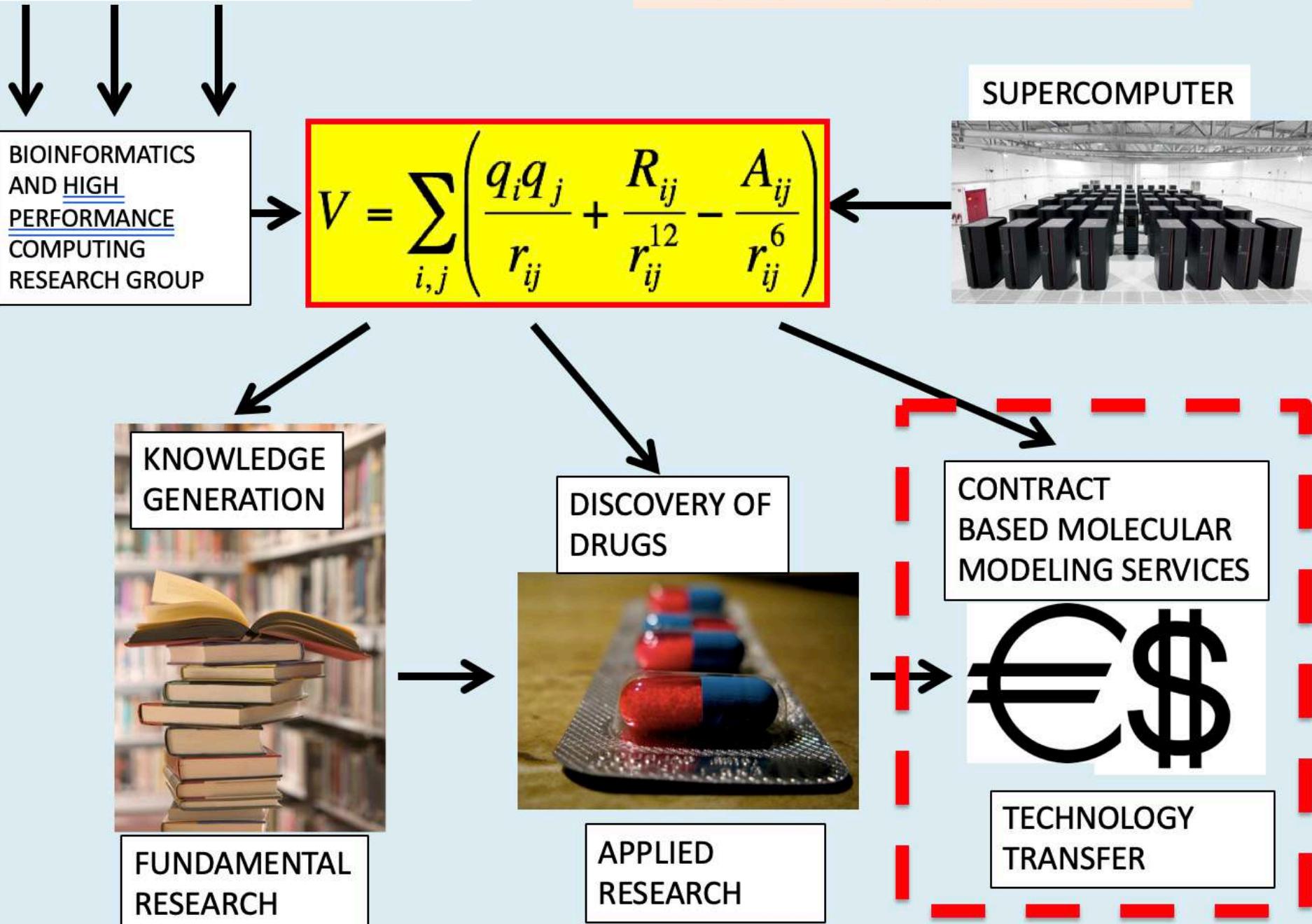
# IN VIVO ZEBRAFISH MODEL

Non invasive



€€€, RESEARCH FUNDS (UCAM)

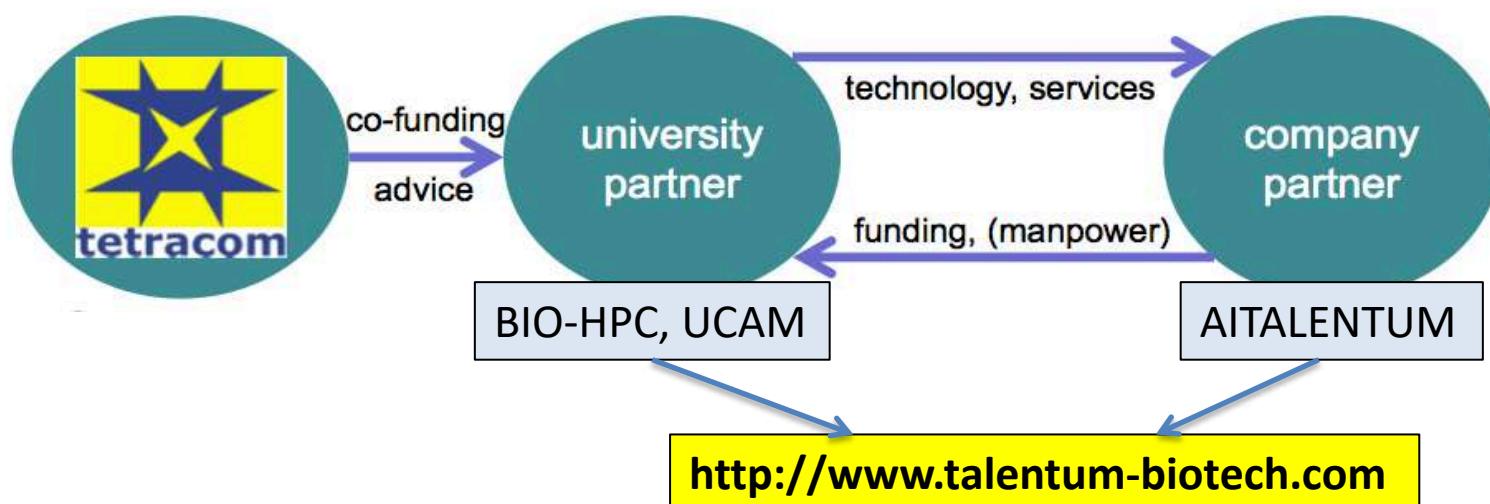
OUR "STARTUP" / "COMPANY"



## ■ Coordination action in EU FP7 (grant agreement no. 609491)

### Technology transfer projects (TTPs)

- TETRACOM's major new instrument
- TTP model:
  - Bilateral project between a university partner U and a company partner C
  - Typical duration: 3-12 months
  - C intends to purchase or license a particular existing technology (e.g. a SW tool or a HW IP block) from U, normally along with some services (porting, training, )
  - TETRACOM provides partial funding



# Acknowledgments



**UCAM**  
UNIVERSIDAD CATÓLICA  
SAN ANTONIO



**intel**  
**f SéNeCa<sup>(+)</sup>**

Agencia de Ciencia y Tecnología  
Región de Murcia



**Villapharma**  
research

**R E S**

RED ESPAÑOLA DE  
SUPERCOMPUTACIÓN



**Barcelona  
Supercomputing  
Center**  
Centro Nacional de Supercomputación



**UCAM**

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DE MURCIA

