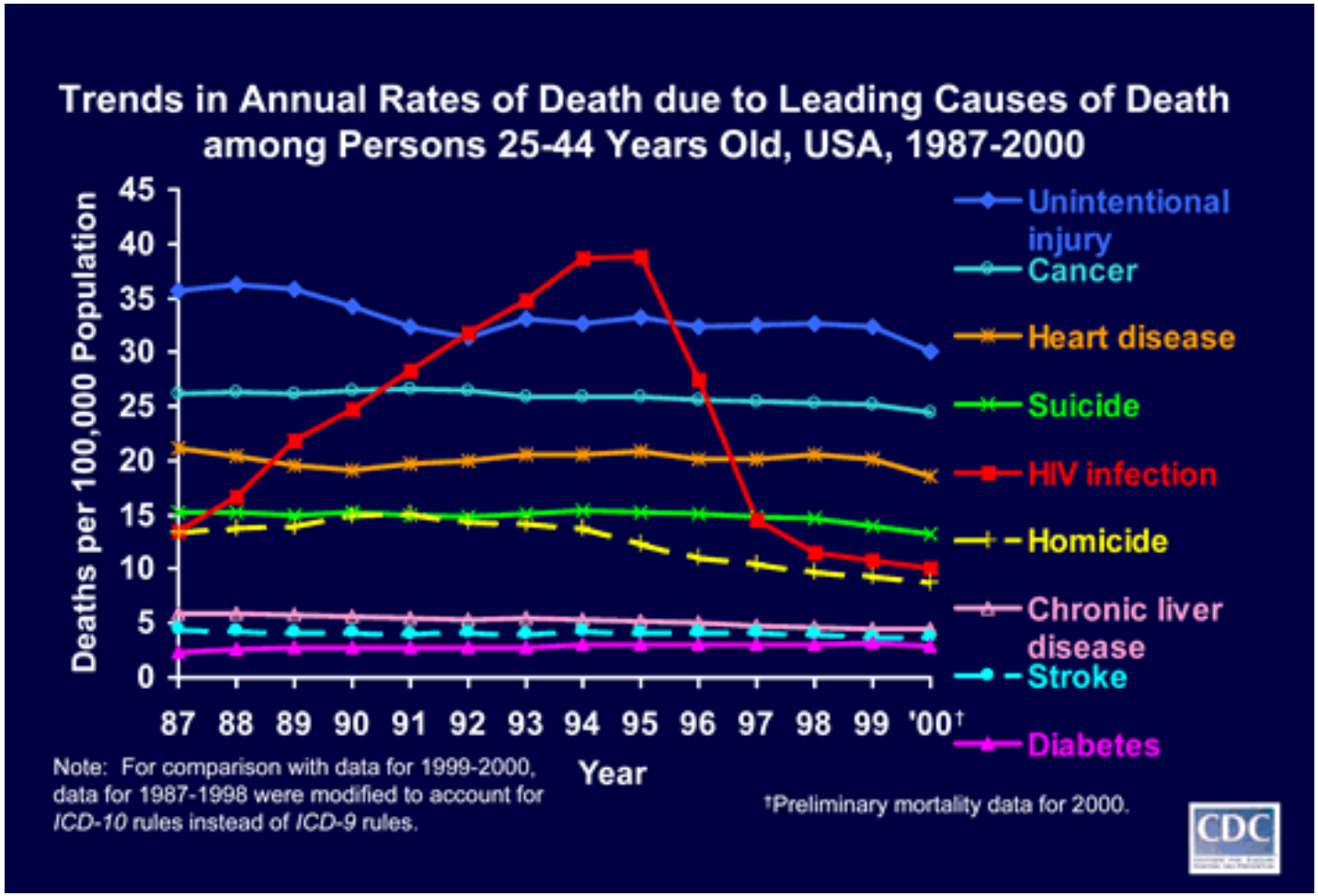

The trend in HIV infection reflects the advances in computer-aided drug design

Anti-viral compounds for HIV therapy whose discovery was aided by computational modelling

1. **Saquinavir:** One of the first protease inhibitors used to treat HIV, Saquinavir was developed using CADD techniques that focused on the structure of the HIV protease enzyme. The drug works by inhibiting this enzyme, which is crucial for the maturation of the HIV virus. **(1995)**
2. **Ritonavir:** Another protease inhibitor, Ritonavir was also developed using CADD. It targets the same HIV protease enzyme as Saquinavir, but with a different structure. Ritonavir is often used in combination with other antiretrovirals due to its ability to boost their effectiveness. **(1996)**
3. **Darunavir:** This drug was designed using CADD to fit into the active site of the HIV protease enzyme more effectively. Darunavir is known for its ability to combat HIV strains resistant to other protease inhibitors. **(2006)**
4. **Raltegravir:** This was the first integrase inhibitor approved for the treatment of HIV. Developed with the aid of CADD, Raltegravir works by inhibiting the HIV integrase enzyme, which is essential for the integration of viral DNA into the DNA of the host cell. **(2007)**
5. **Dolutegravir:** Similar to Raltegravir, Dolutegravir is an integrase inhibitor developed using CADD. It has a high barrier to resistance and is effective against some HIV strains that are resistant to Raltegravir. **(2013)**
6. **Tenofovir Alafenamide (TAF):** This is a prodrug of Tenofovir, developed using CADD to improve the drug's stability and uptake into cells. TAF is more efficient at delivering the active drug to HIV-infected cells, leading to lower doses and reduced side effects. **(2015)**
7. **Etravirine:** A non-nucleoside reverse transcriptase inhibitor (NNRTI), Etravirine was developed using CADD to address resistance issues seen in first-generation NNRTIs. It works by binding to and inhibiting the reverse transcriptase enzyme, which is crucial for the replication of the HIV virus. **(2008)**

Q: “Is there really a case where a drug that’s on the market was designed by a computer ?”

A: “No”

Jorgensen, WL (2004) “The many roles of computation in drug discovery” *Science* 303:813

Computational modelling techniques cannot *per se* finding new drugs (not yet, at least), but they are na immensely and indispensable tool in the processo of drug discovery!

A Simulação Computacional pode **guiar** o processo de síntese e optimização de compostos…

Journal of Medicinal Chemistry

Article

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Computationally-Guided Optimization of a Docking Hit to Yield Catechol Diethers as Potent Anti-HIV Agents

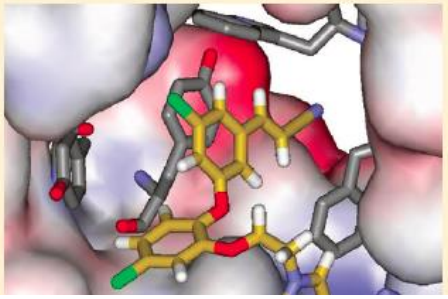
Mariela Bollini,[†] Robert A. Domaoal,[‡] Vinay V. Thakur,[†] Ricardo Gallardo-Macias,[†] Krasimir A. Spasov,[‡] Karen S. Anderson,*[,‡] and William L. Jorgensen*[,†]

[†]Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

[‡]Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520-8066, United States

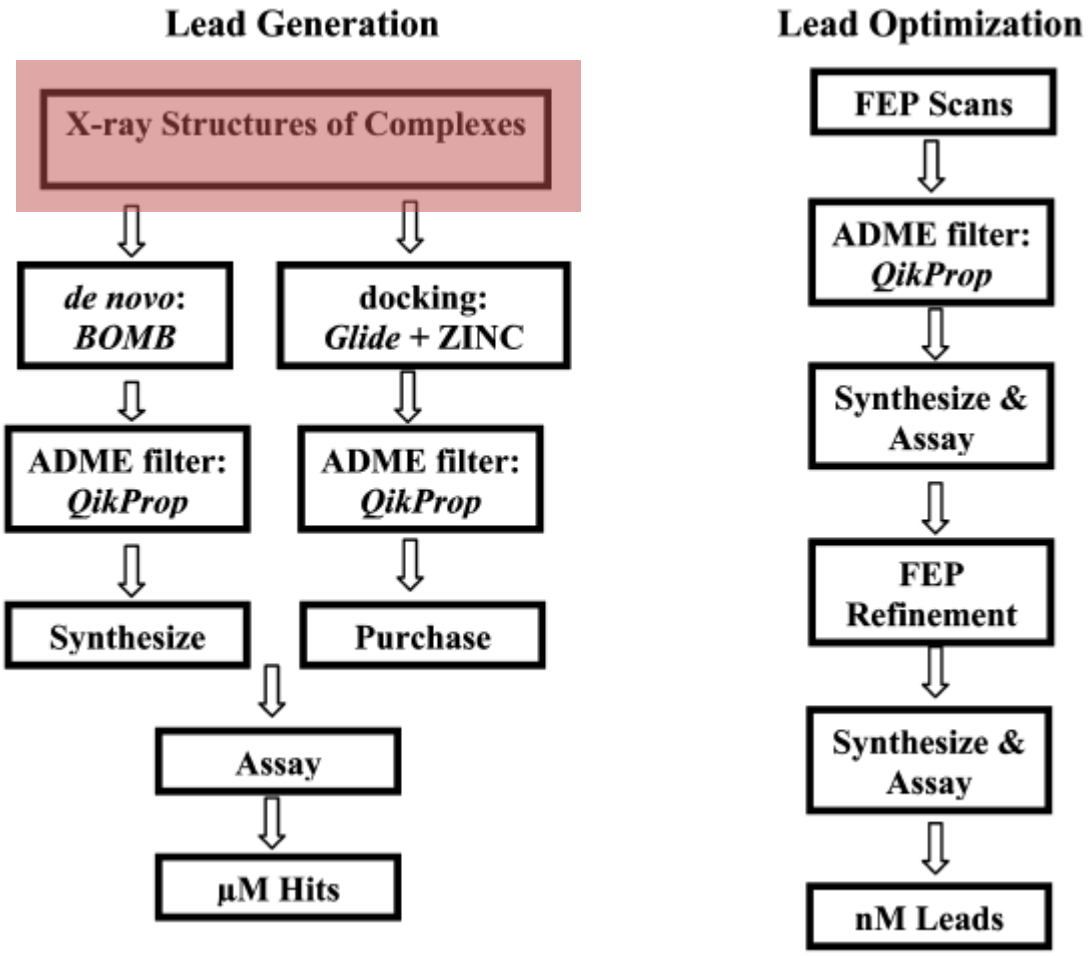
Supporting Information

ABSTRACT: A 5-μM docking hit has been optimized to an extraordinarily potent (55 pM) non-nucleoside inhibitor of HIV reverse transcriptase. Use of free energy perturbation (FEP) calculations to predict relative free energies of binding aided the optimizations by identifying optimal substitution patterns for phenyl rings and a linker. The most potent resultant catechol diethers feature terminal uracil and cyanovinylphenyl groups. A halogen bond with Pro95 likely contributes to the extreme potency of compound 42. In addition, several examples are provided illustrating failures of attempted grafting of a substructure from a very active compound onto a seemingly related scaffold to improve its activity.



Bollini M (2011) *J Med Chem* 54:8582

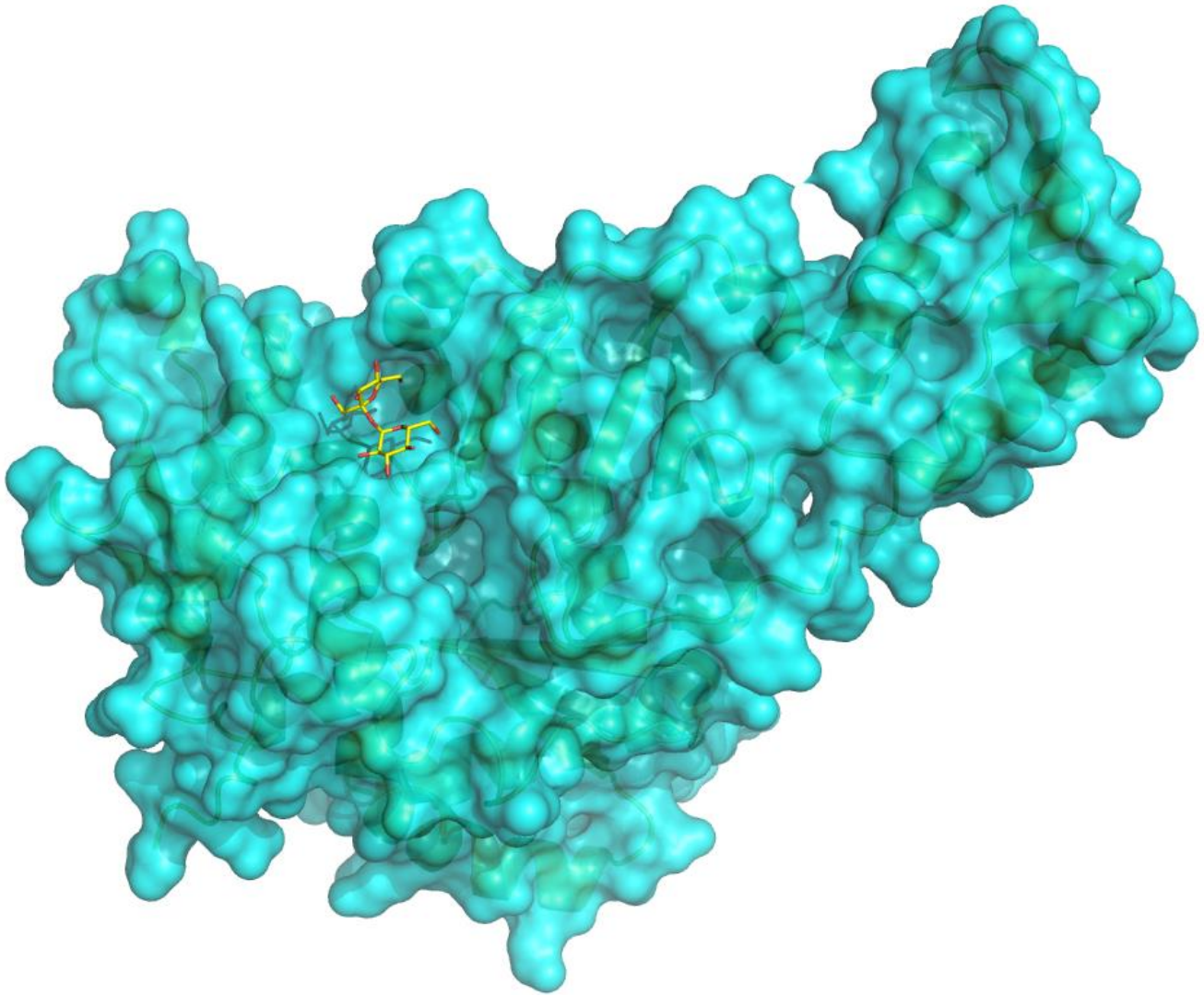
Pipeline para gereação e optimização computacional de *leads*



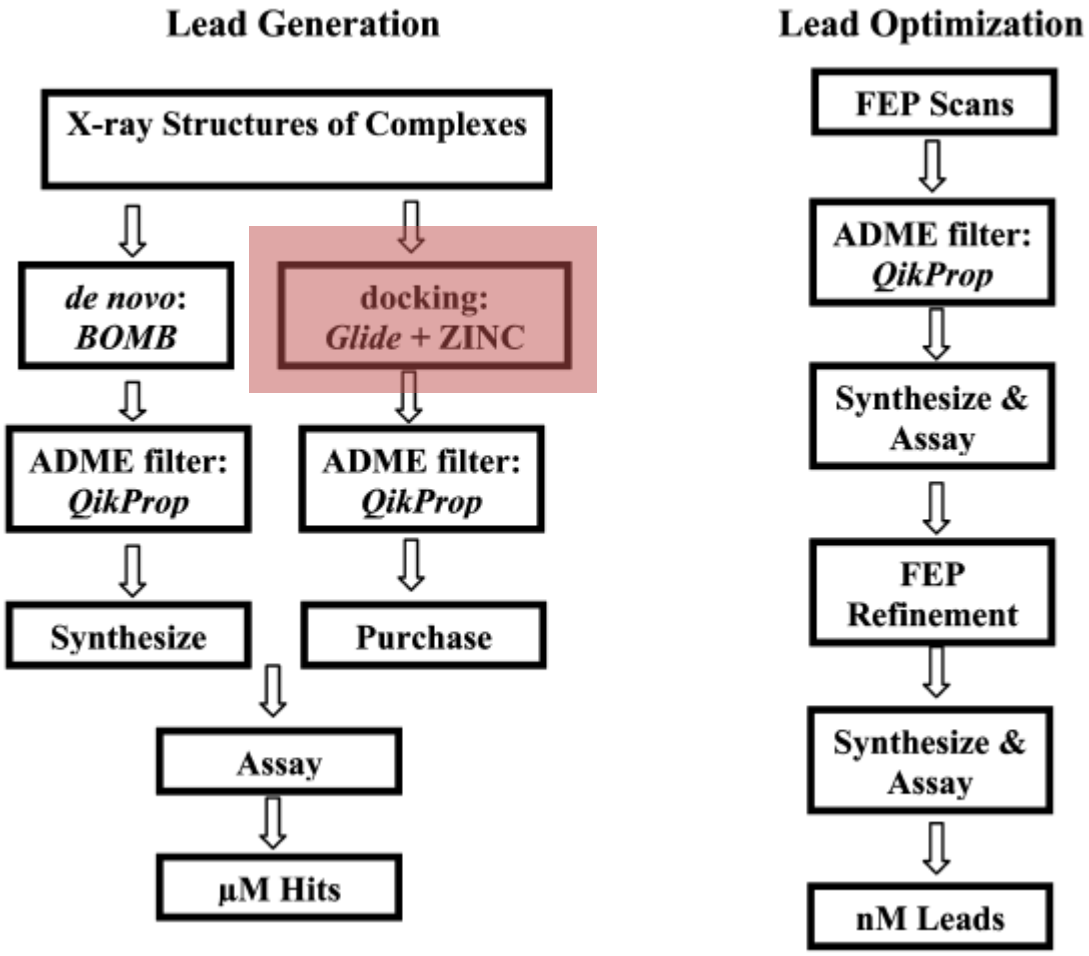
µM hit ⇨ nM lead

Jorgensen, WL (2009) *Acc Chem Res* 42:724

Transcritase reversa do HIV (HIV-RT)



Pipeline para gereação e optimização computacional de *leads*

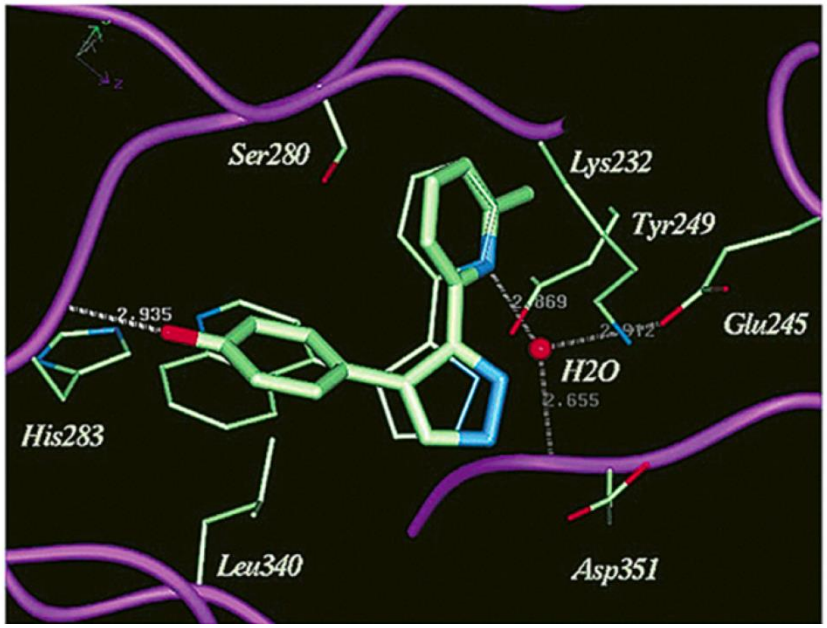


µM hit ⇨ nM lead

Jorgensen, WL (2009) *Acc Chem Res* 42:724

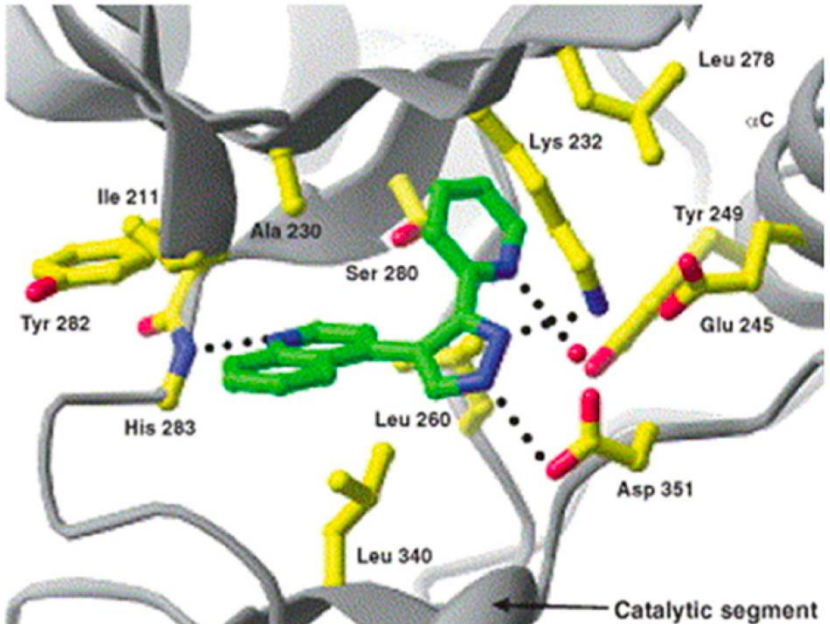
Virtual screening can produce the same results as real screening

I)



High-troughput screening

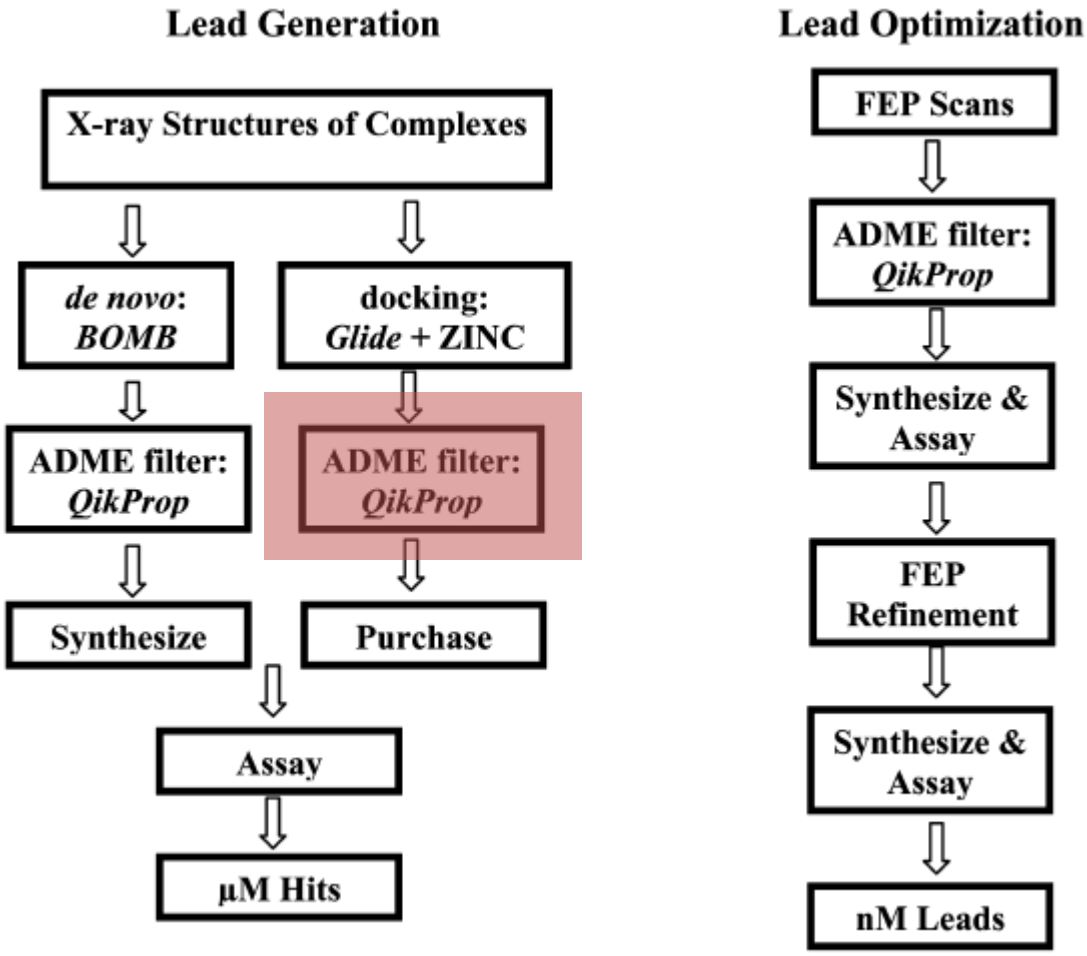
II)



Virtual screening

TbR-I kinase domain

Pipeline para gereação e optimização computacional de *leads*



µM hit ⇨ nM lead

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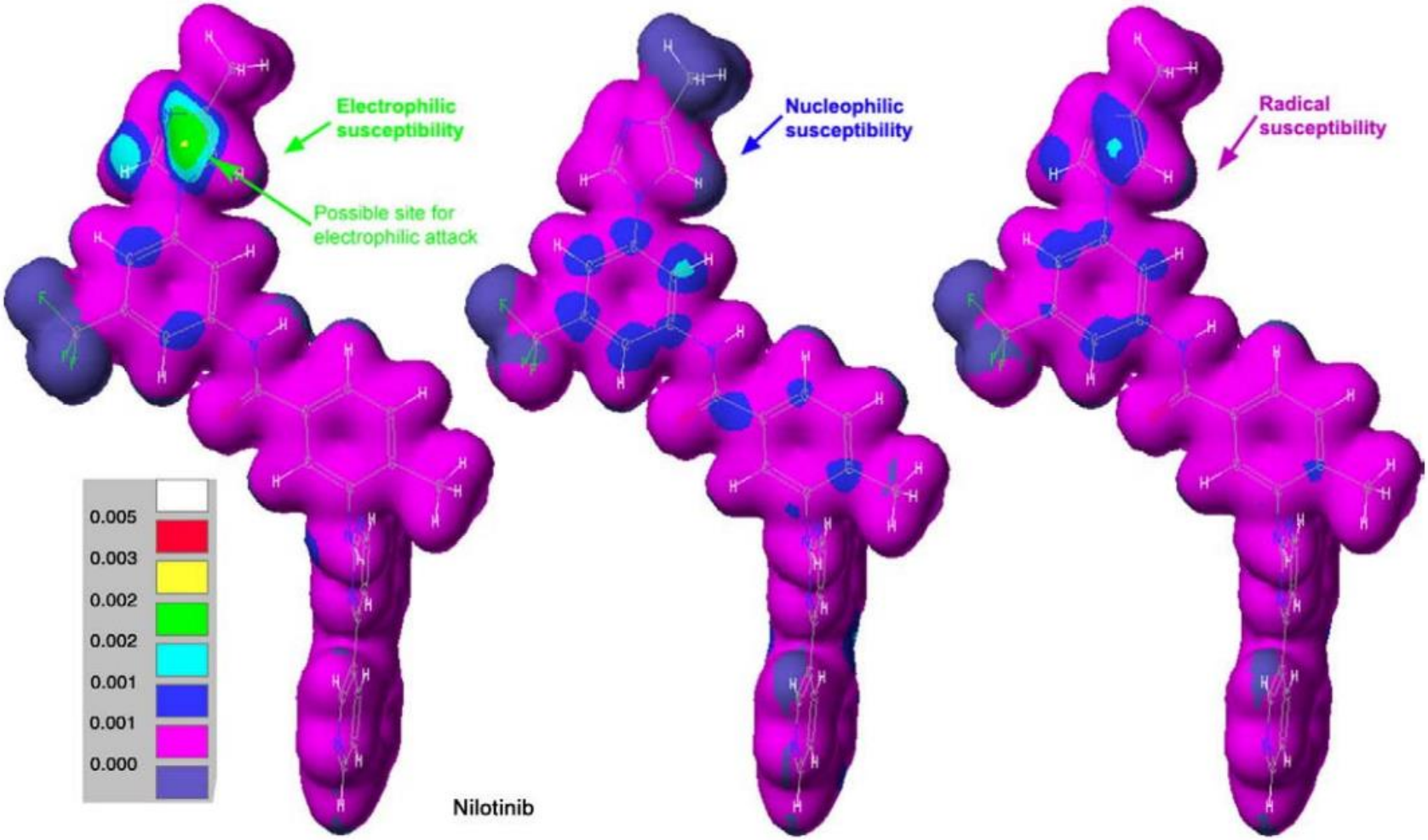
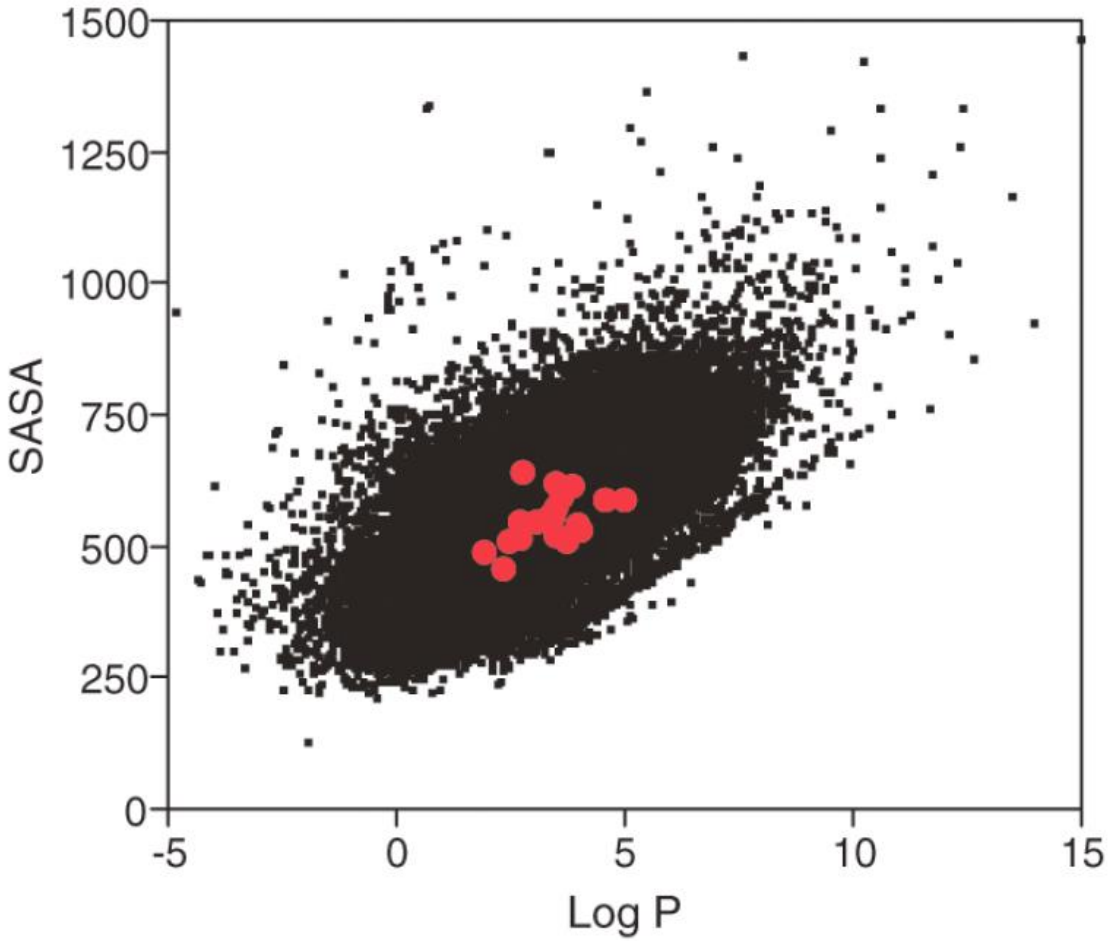
Análise das propriedades moleculares



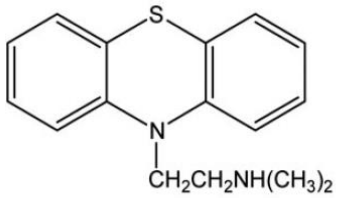
Filtragem do espaço de busca



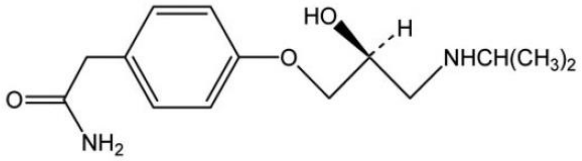
Os pontos vermelhos representam vários inibidores da HIV-1 RT (revelam semelhança na área e na polaridade)

Jorgensen, WL (2004) *Science* 303:813

Previsão de propriedades ADMET



chlorpromazine

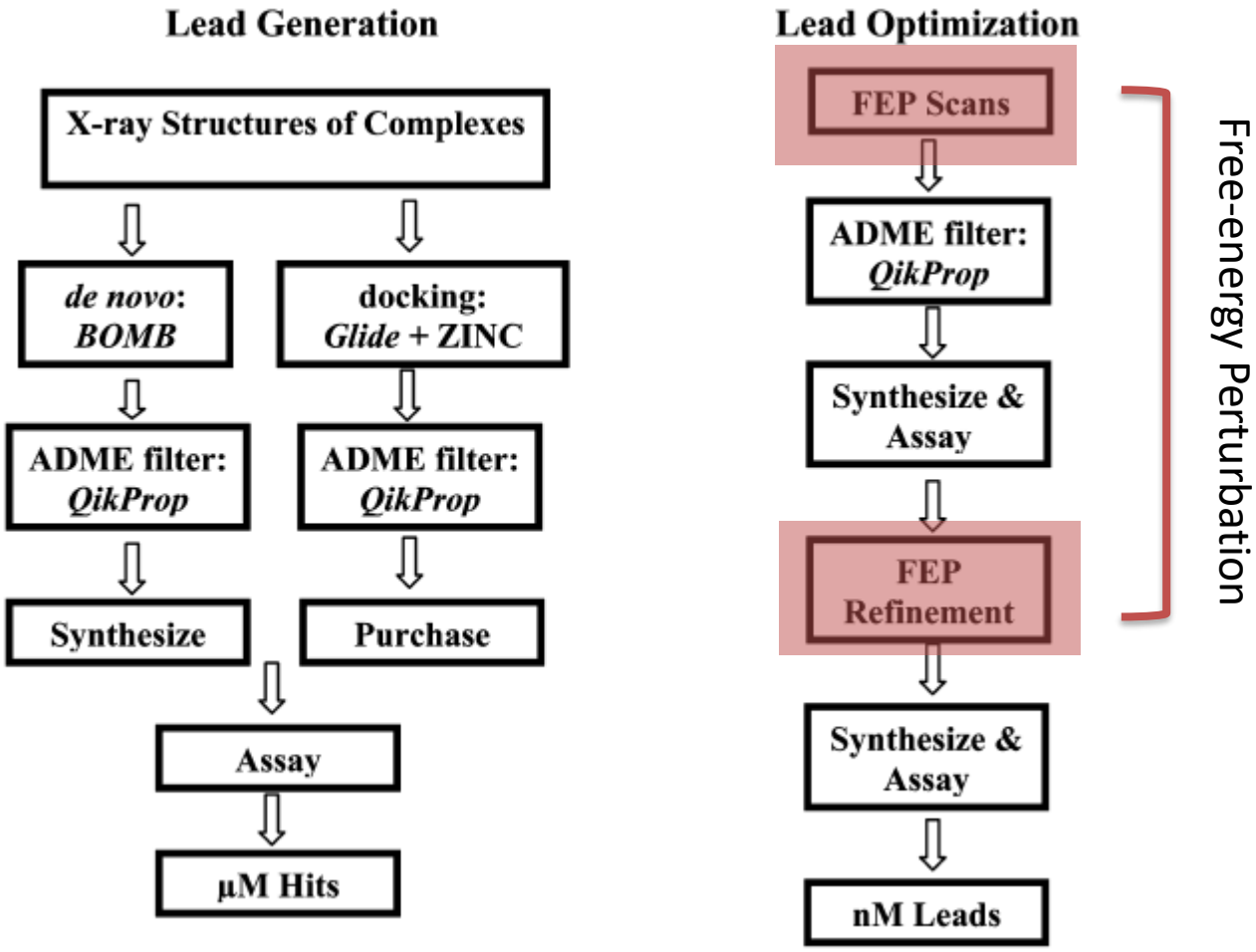


atenolol

Table 1. Predicted and experimental (in parentheses) ADME-related properties.

	Chlorpromazine	Atenolol
log S	–4.5 (–5.01)	–0.61 (–1.30)
log P	4.80 (5.19)	0.40 (0.16)
log BB	0.74 (1.06)	–1.09
log K_{hsa}	0.78 (1.10)	–0.79 (–0.48)
PCaco (nm/s)	2003	66 (33)
PMDCK (nm/s)	1425	33 (18)
CNS Activity	++	––

Pipeline para gereação e optimização computacional de *leads*



µM hit ⇨ nM lead

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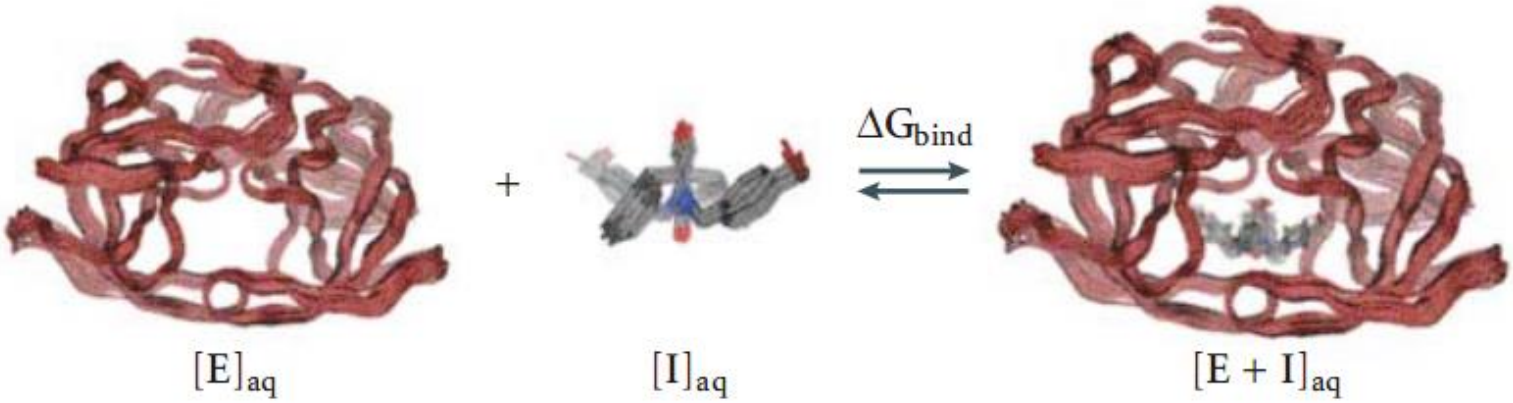
Prediction of binding efficiency is crucial

$$[EI]_{aq} \rightleftharpoons [E]_{aq} + [I]_{aq} \qquad (1)$$

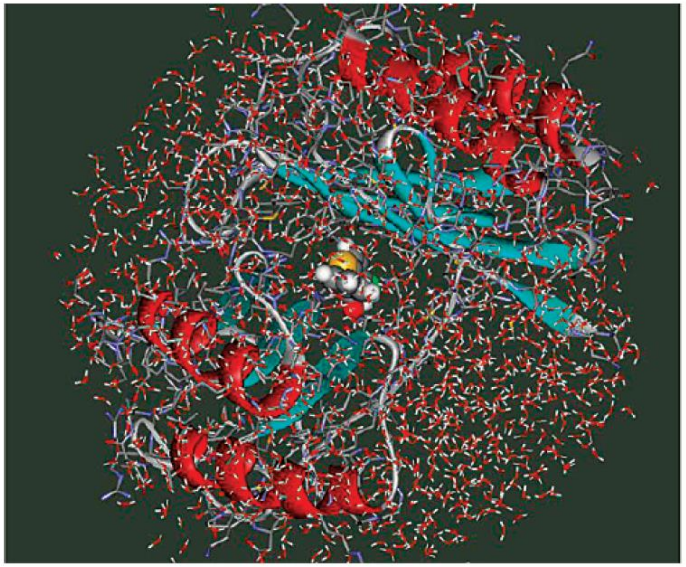


$$\Delta G = -RT\ln K_A$$

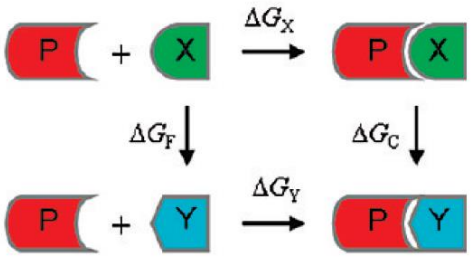
$$K_A = K_i^{-1} = \frac{[EI]}{[E][I]}$$

K_A – association constant

K_I , K_d – dissociation constant

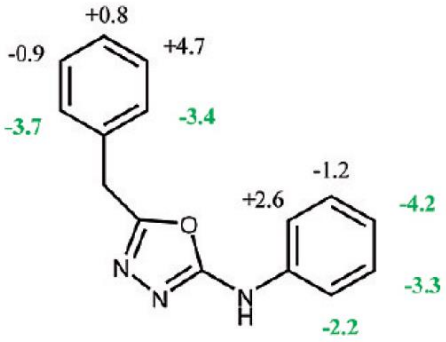


FEP (Free-energy perturbation)

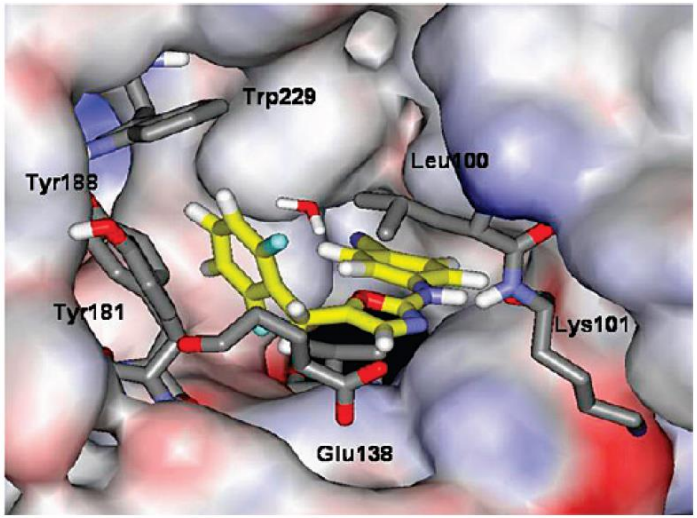


Ciclo termodinâmico para o cálculo da ernergia relativa de binding ($\Delta\Delta G_{bind}$)

Energias calculadas por FEP para a substituição dos hidrogénios indicados por átomos de **Cloro**.



$\Delta\Delta G_{binding}$ (kcal/mol)



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Previsão teórica de constantes de dissociação para guiar o processo de descoberta de leads

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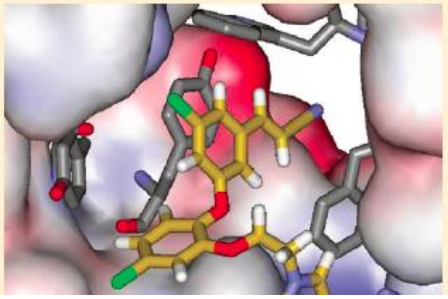

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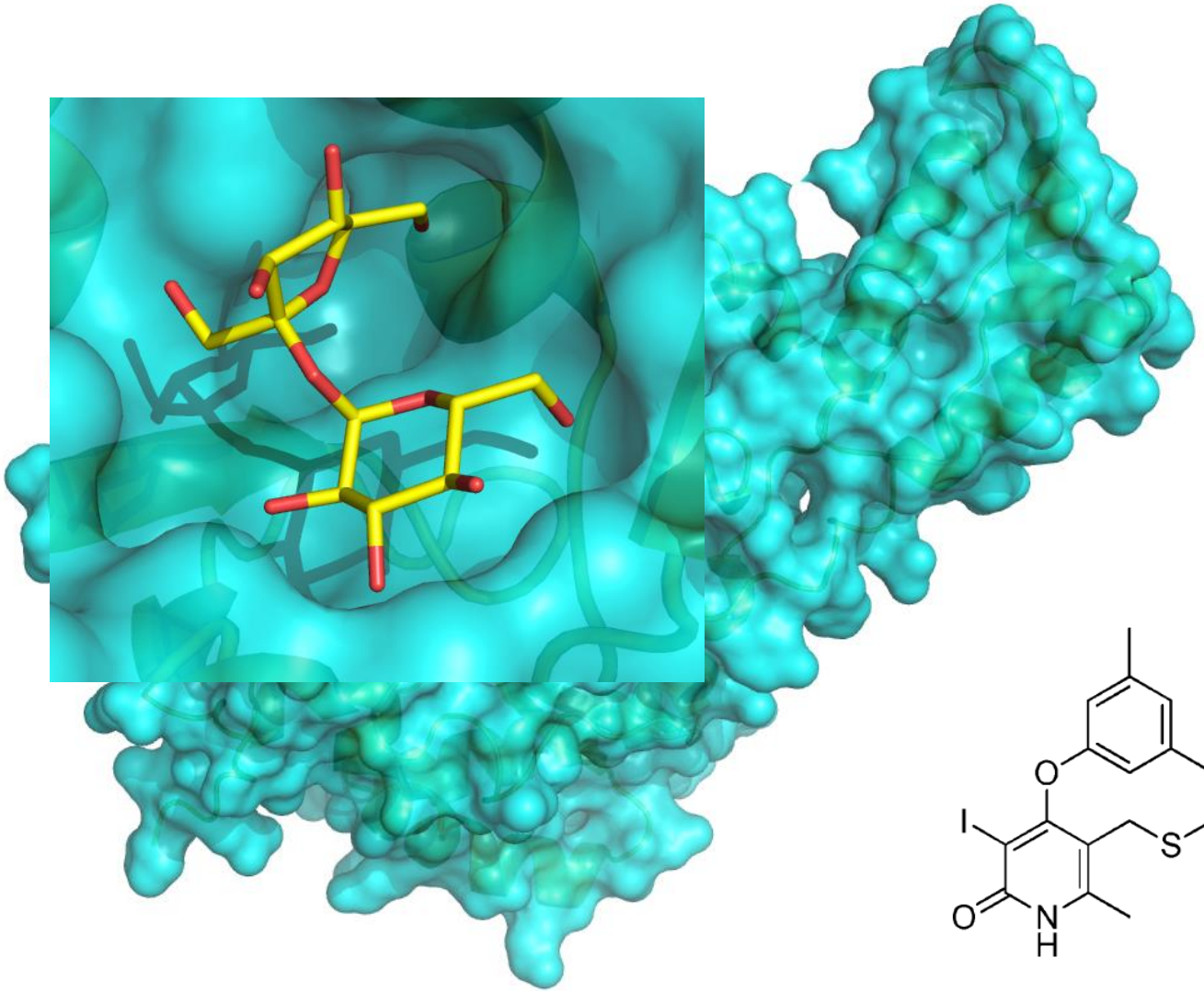
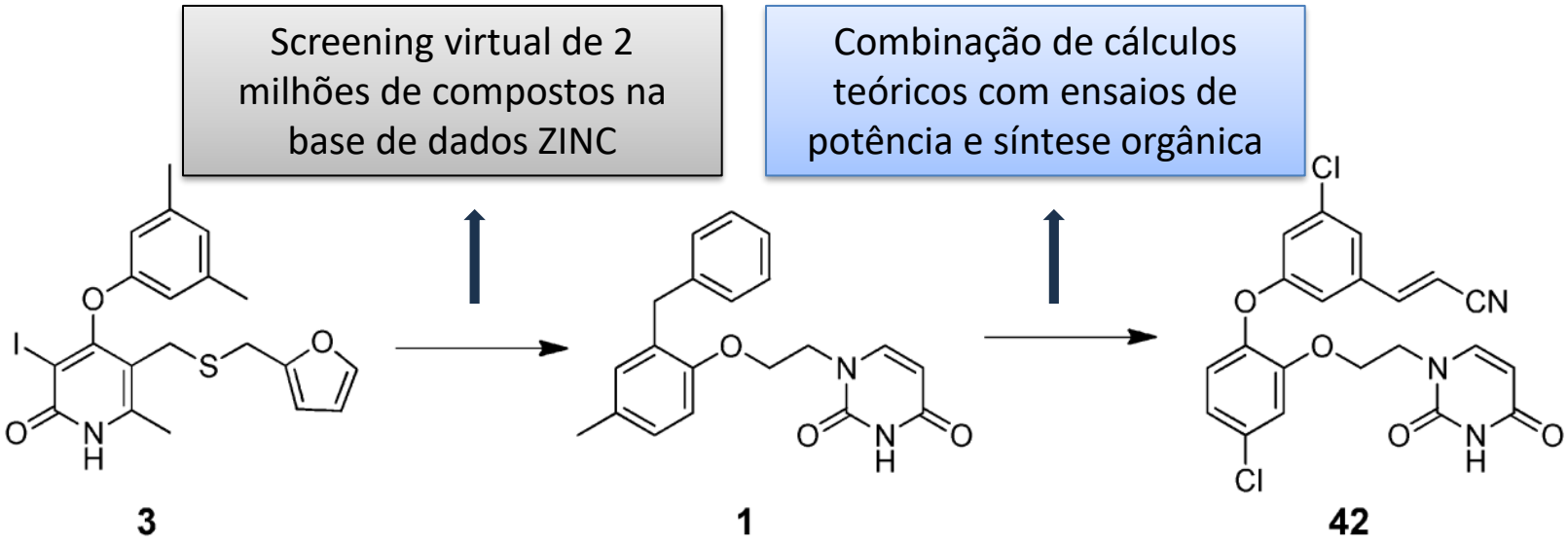
HIV reverse transcriptase (HIV-RT)



3

Previsão teórica de constantes de dissociação para guiar o processo de descoberta de leads



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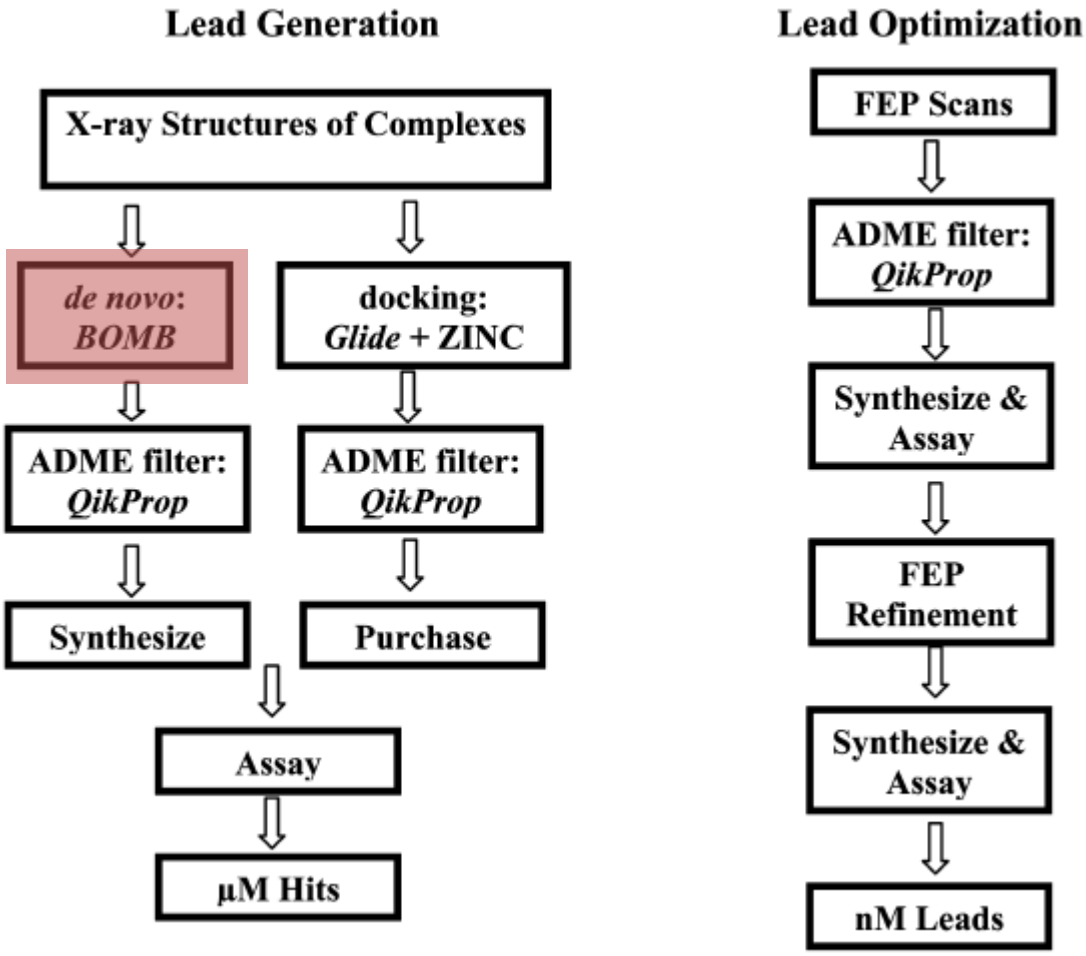
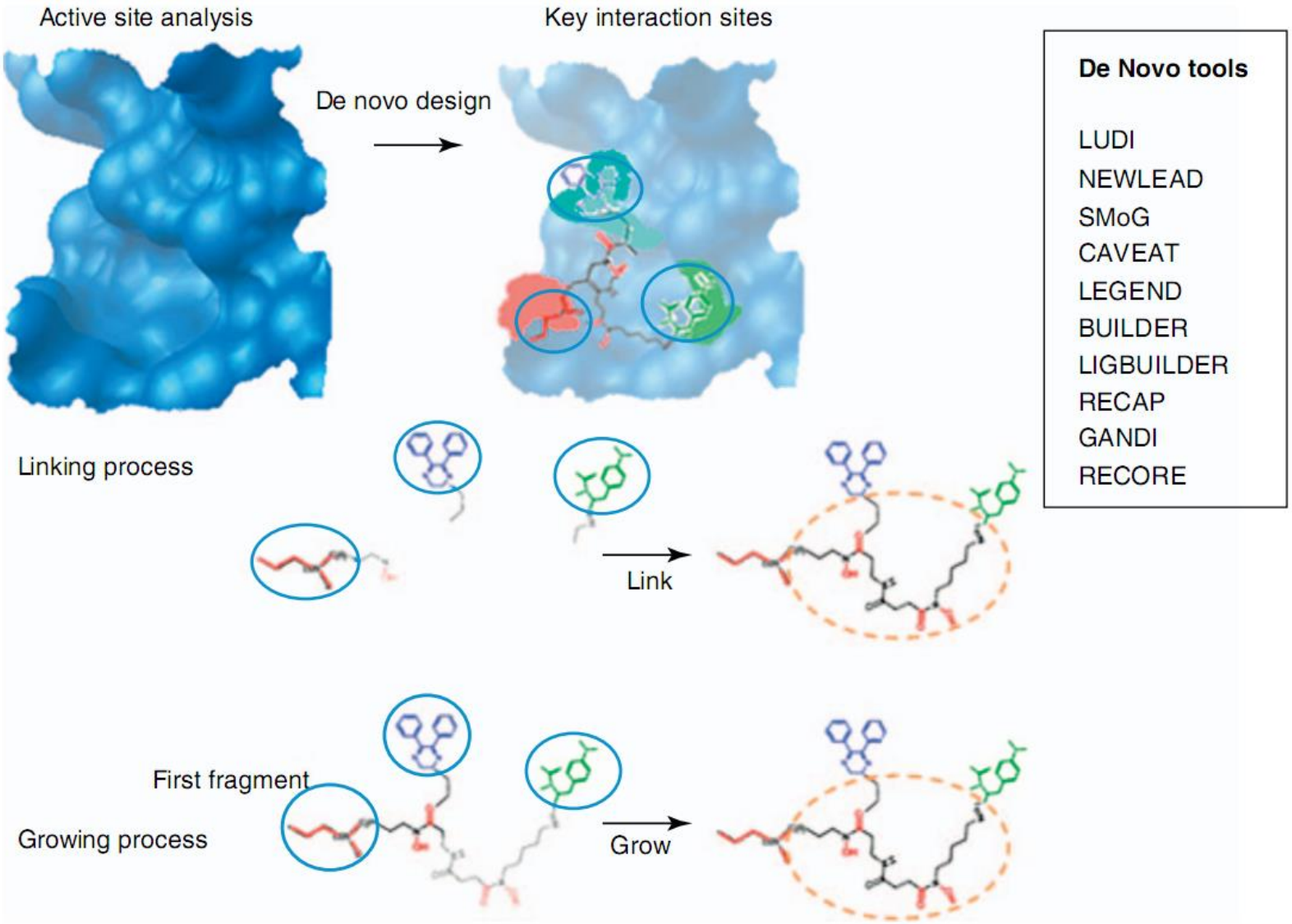
Pipeline para gereação e optimização computacional de *leads*



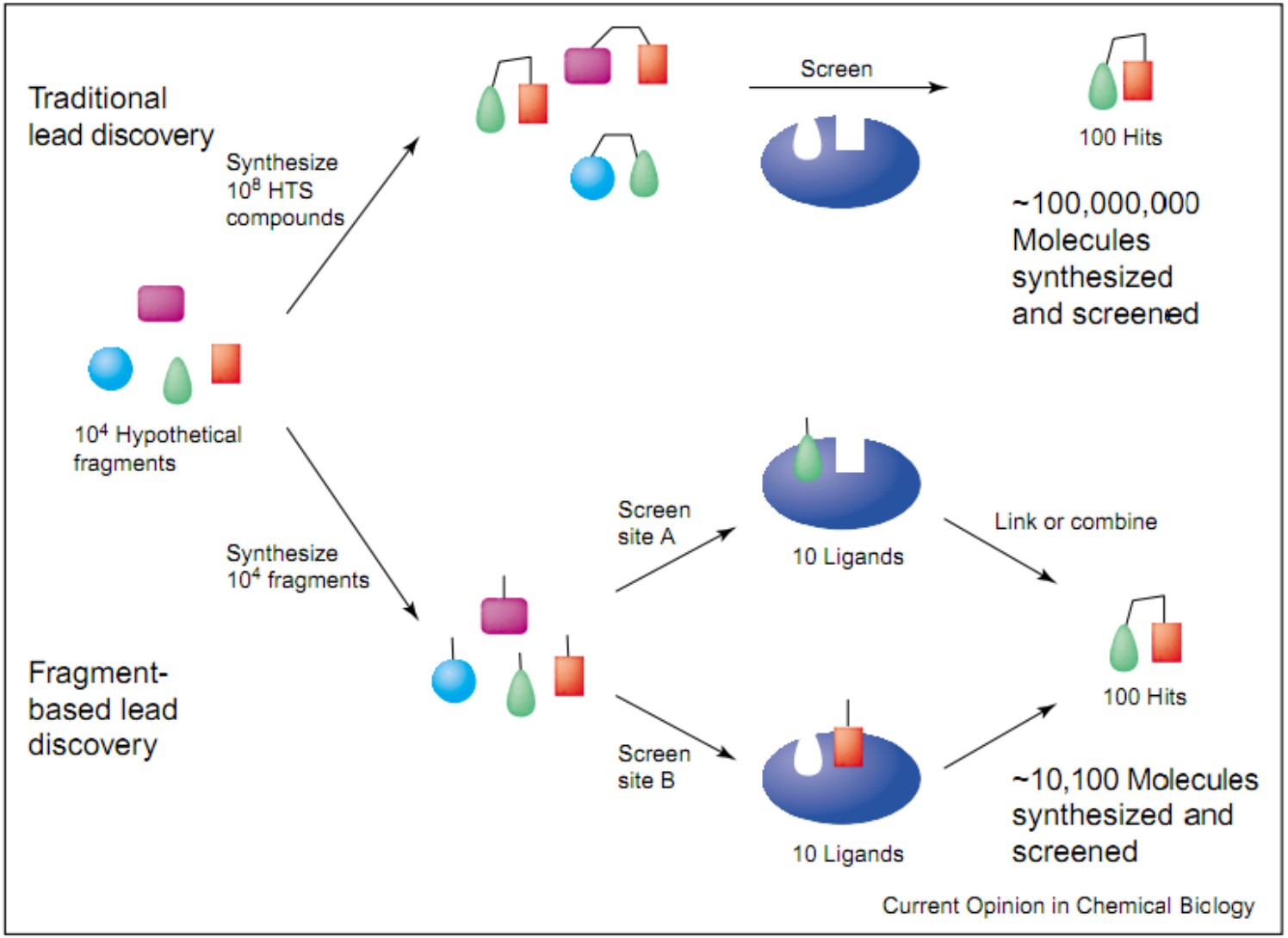
µM hit ⇨ nM lead

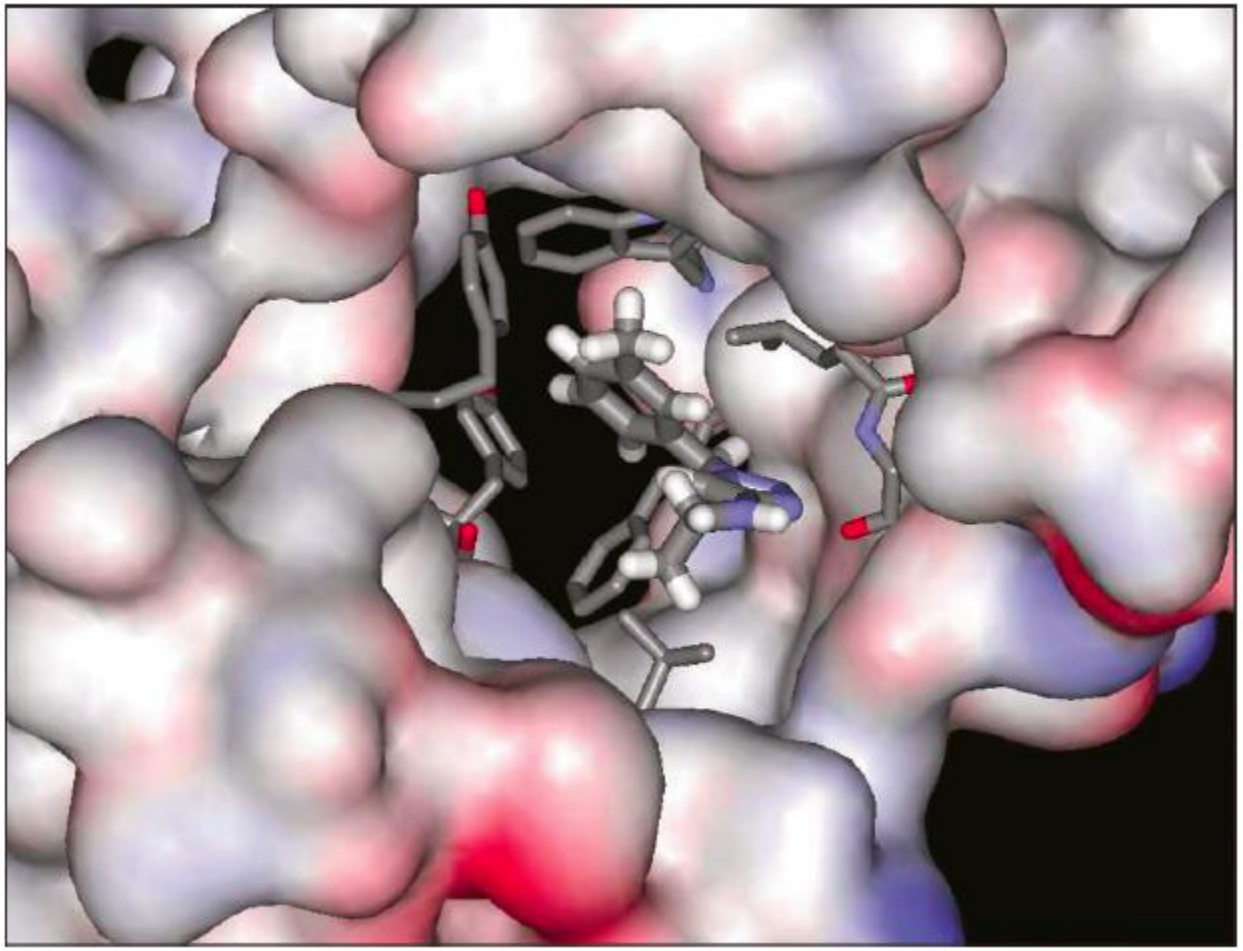
Jorgensen, WL (2009) *Acc Chem Res* 42:724

Desenho virtual de fármacos *de novo*



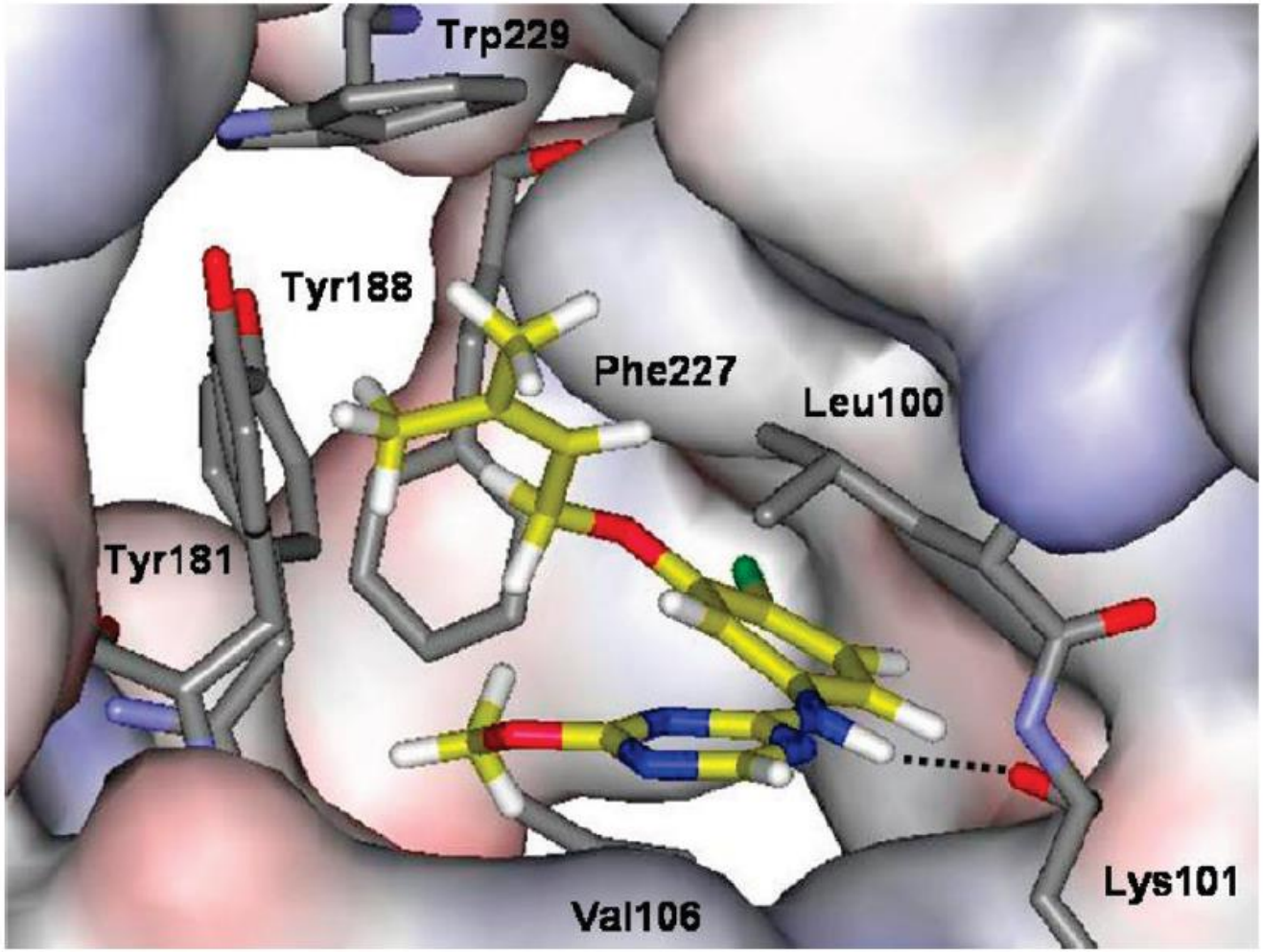
Pesquisa de fragmentos *versus* técnicas tradicionais





HIV-RT inhibitor built using BOMB by growing from na ammonia molecule on the active site.

Jorgensen, WL (2009) *Acc Chem Res* 42:724



Complex of HIV-RT with a non-nucleoside inhibitor (NNRTI) built using *BOMB*.. The hydrogen bond with the oxygen atom of Lys101 is dashed

Jorgensen, WL (2009) *Acc Chem Res* 42:724

HIV-RT inhibitors whose discovery was helped by the computational work of Prof. Jorgensens group

1. **Rilpivirine (Edurant)**: Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used in the treatment of HIV-1 infection. Professor Jorgensen's group played a role in its development through their work on the computational design and optimization of NNRTIs. Their research contributed to the understanding of how these drugs bind to the reverse transcriptase enzyme and how their structures could be optimized for better efficacy and reduced resistance.

2. **Doravirine (Pifeltro):** Another NNRTI, Doravirine was approved for the treatment of HIV-1 infection. The Jorgensen group's work in the field of computational chemistry and drug design provided valuable insights into the structure-activity relationships of NNRTIs, which was crucial in the development of drugs like Doravirine.

Professor Jorgensen's group is known for their pioneering work in the field of computer-aided drug design, particularly in the development of predictive models and methods for the calculation of free energies in drug-receptor interactions. Their work has been instrumental in improving the efficiency of drug discovery processes, leading to the development of more effective and safer drugs.

Desenho de fármacos: racional *versus* "irracional"

- Abordagem tradicional: empiricismo, intuição, medicina tradicional, pesquisa de força bruta (High-throughput screening)

- Desenho racional: busca de moléculas com características físico-químicas adequadas à actuação sobre o mecanismo da doença e à eficiente absorção/metabolização/eliminação

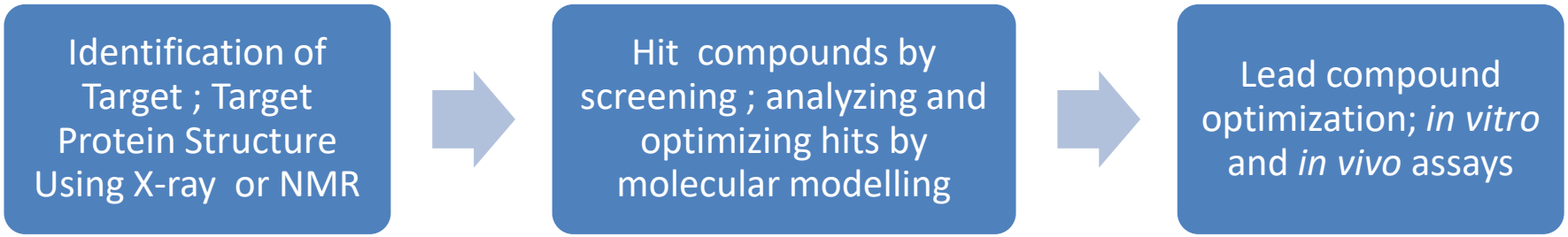
Irrational drug design approach

Natural product collection → Random observation of effect; brute force screening → Purification of active compound; chemical modification to enhance properties

Salix alba

Example: The bark of the willow tree has been known since ancient times for its analgesic and anti-piretic properties. In the XIX century, chemists identified salicylic acid as the active compound of the willow extract and made several chemical modifications to it, including acetylation. In 1897, chemists from Bayer recognized acetylsalicylic acid as a less-irritating alternative to the already know salicylate plant extracts. Bayer marketed the substance in 1999 under the name Aspirin and began selling it worldwide.

Rational drug design approach

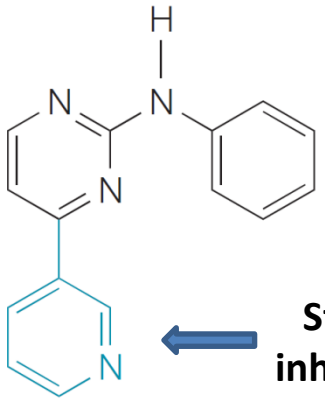


Example: The drug *Gleevec* for the treatment of Chronic Myelogenous Leukemia (CML) was found by screening a library of compounds against Protein Kinase C active. Compounds that showed activity against PKC were chemical modified based on computational modelling studies and X-ray structures of their interaction with the target for CML, the BCR-ABL kinase. The molecules were modified until a compound with sufficient inhibitory potency against BCR-ABL, safety and bioavailability was found.

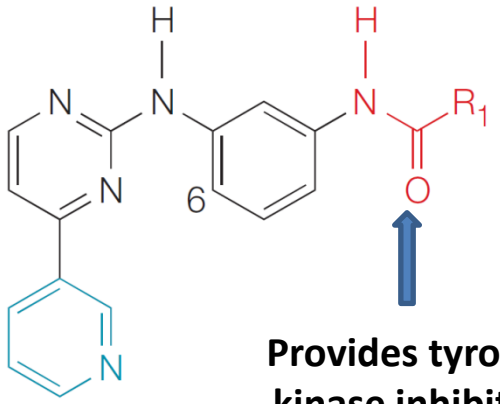
Rational design of Glivec (Imatinib)

Chronic Mielogenous Leucemia

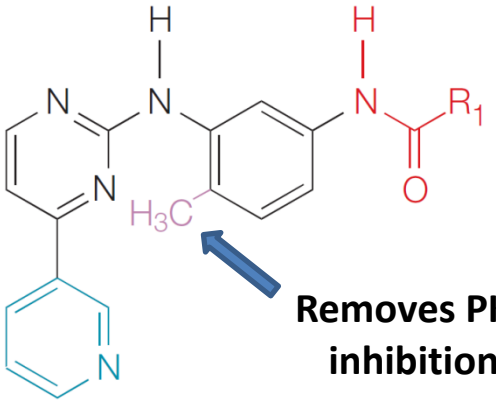
Found on a screen against PKC activity:



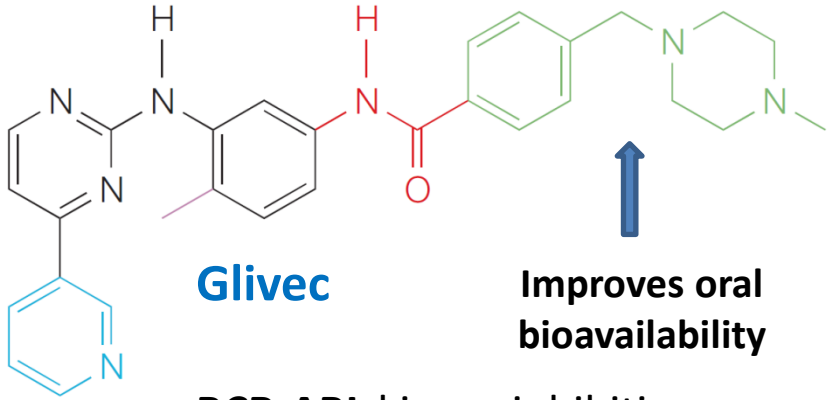
Strong inhibition against PKC



Provides tyrosine kinase inhibition



Removes PKC inhibition



Glivec

Improves oral bioavailability

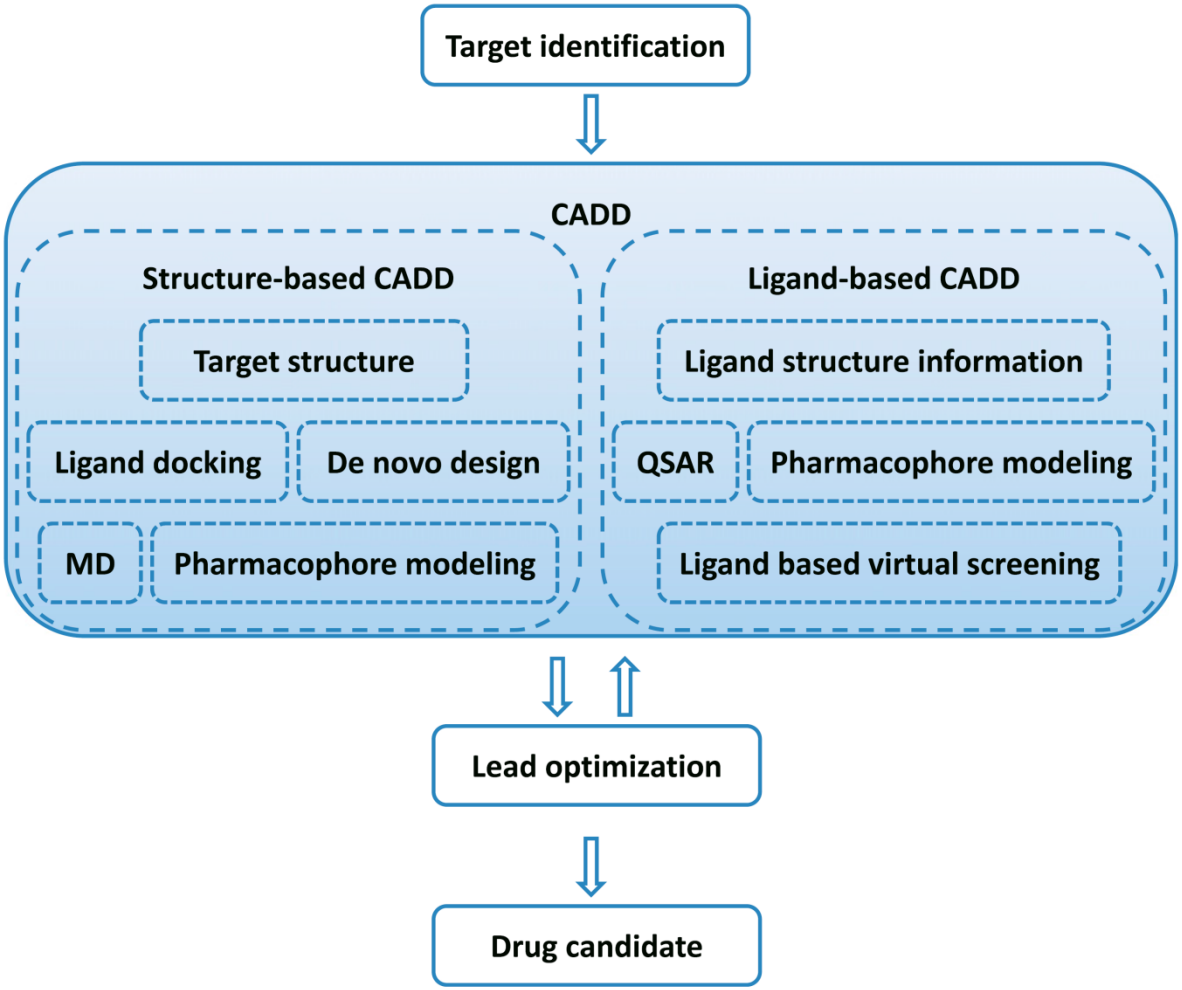
BCR-ABL kinase inhibition

Structure *versus* Ligand-Based Drug Design

	Known Ligands	Unknown Ligands
Know protein structure	**Structure-based drug design (SBDD)** Protein modelling Docking-guided Chemical optimization	***De novo* design**
Unknown protein structure	**Ligand-based drug design (LBDD)** *1 or more ligands* • Similarity searching *Several ligands* • Pharmacophore searching *Many ligands (20+)* • Quantitative Structure-Activity Relationships (QSAR)	**No rational approach** Need experimental data of some sort Can apply ADMET filters

ADMET: absorption, distribution, metabolism, excretion, toxicity

CADD in drug discovery/design pipeline

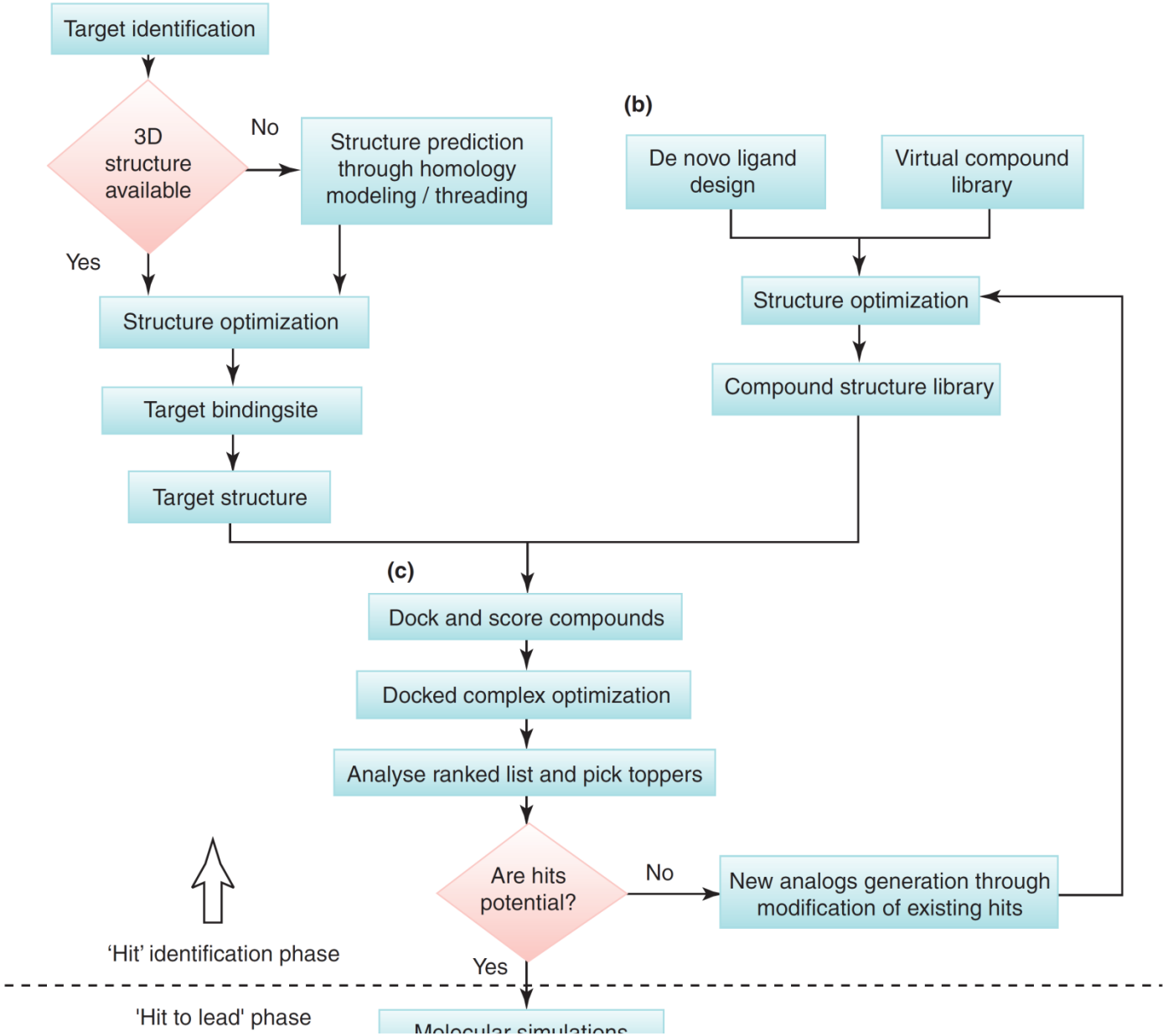


CADD – Computer-Aided Druge Design

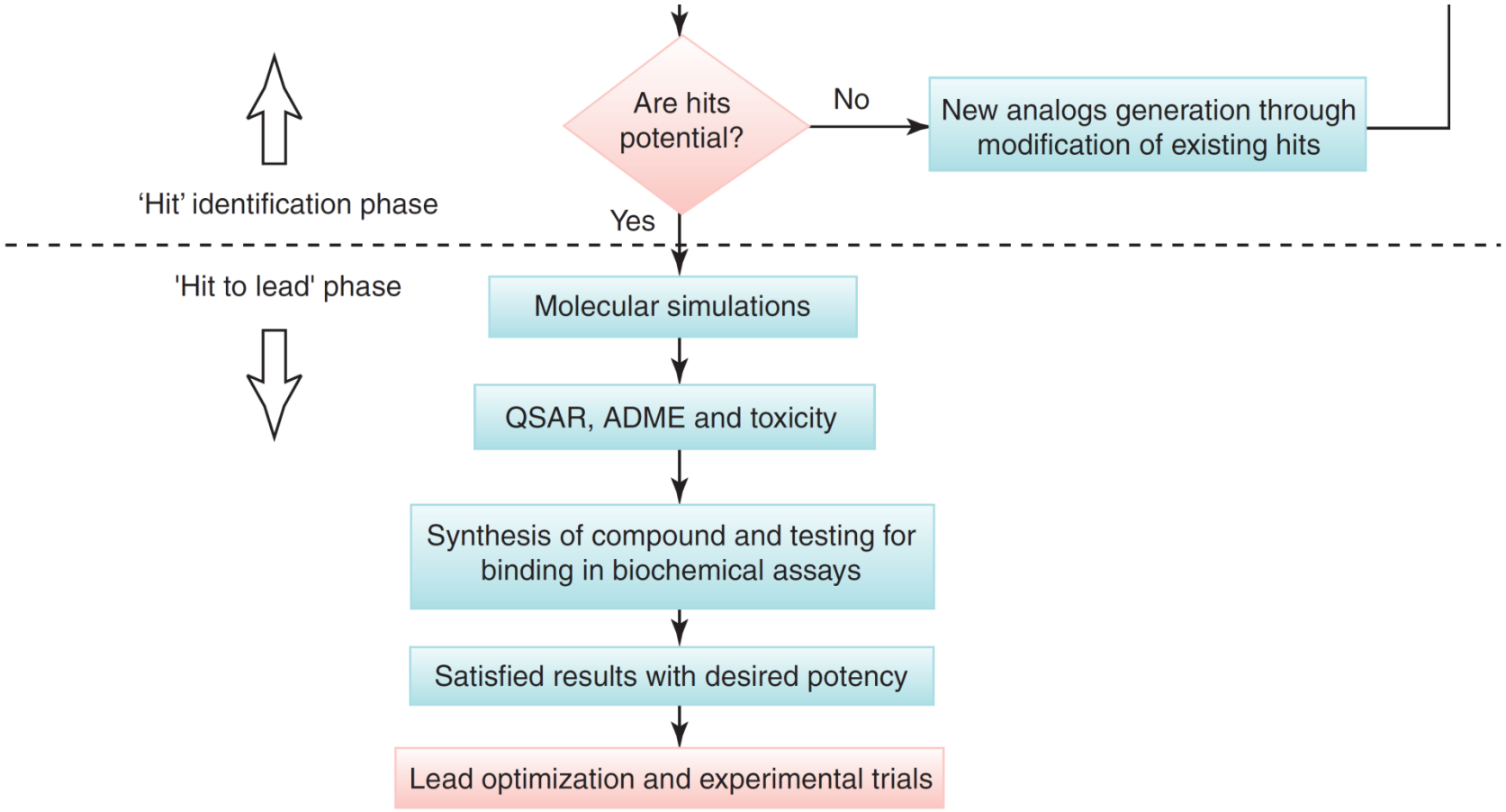
Desenho de fármacos baseado em estrutura

- O SBDD tornou-se possível com o desenvolvimento de uma vasta biblioteca de estruturas de receptores e enzimas
- Neste tipo de abordagem a forma e características electrónicas do centro activo são consideradas desde o início
- A estruturas cristalográficas da proteína alvo e do ligando são determinadas experimentalmente permitindo obter informação sobre as interacções do complexo
- Com base na informação estrutural procura-se encontrar as modificações que optimizem a interacção do ligando com o receptor
- Optimização da potência, afinidade e selectividade, preservando as propriedades ADMET !

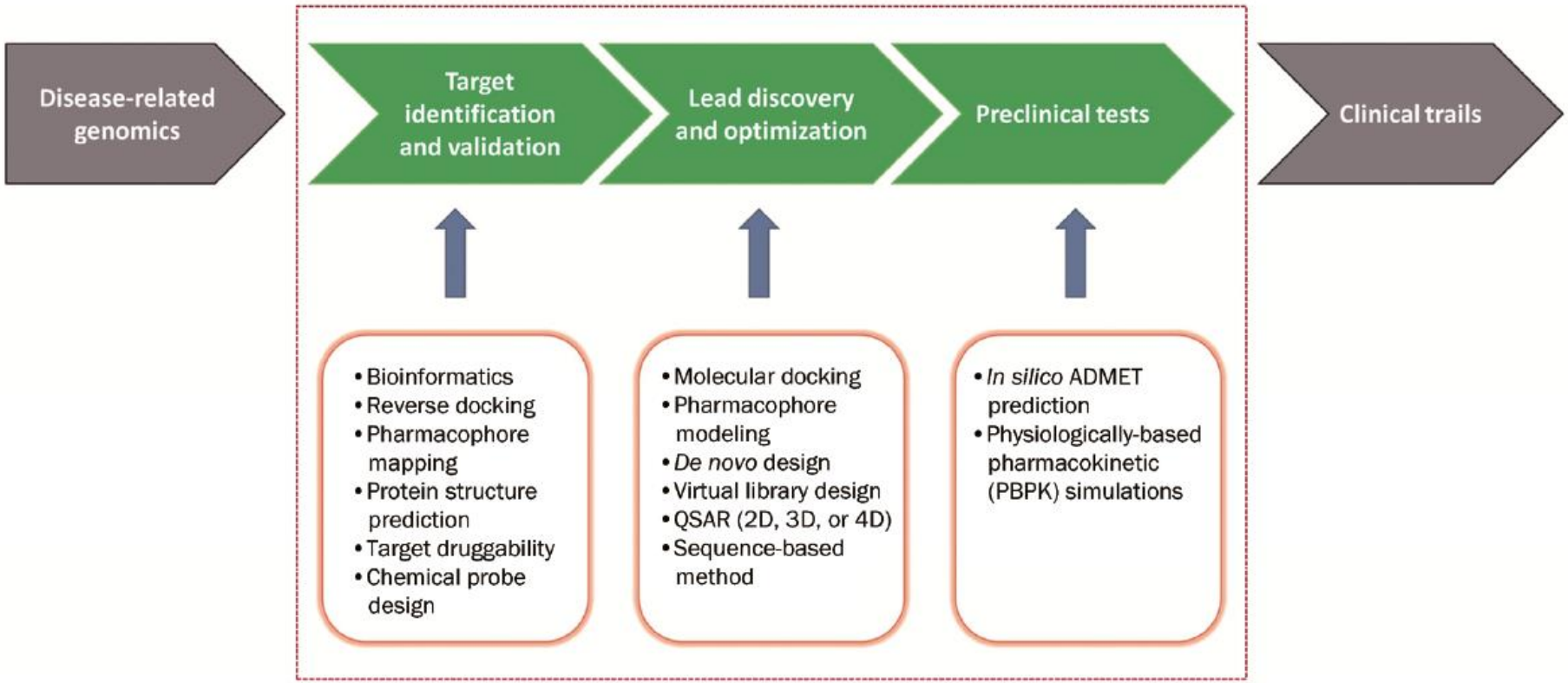
SBDD – Hit Identification phase

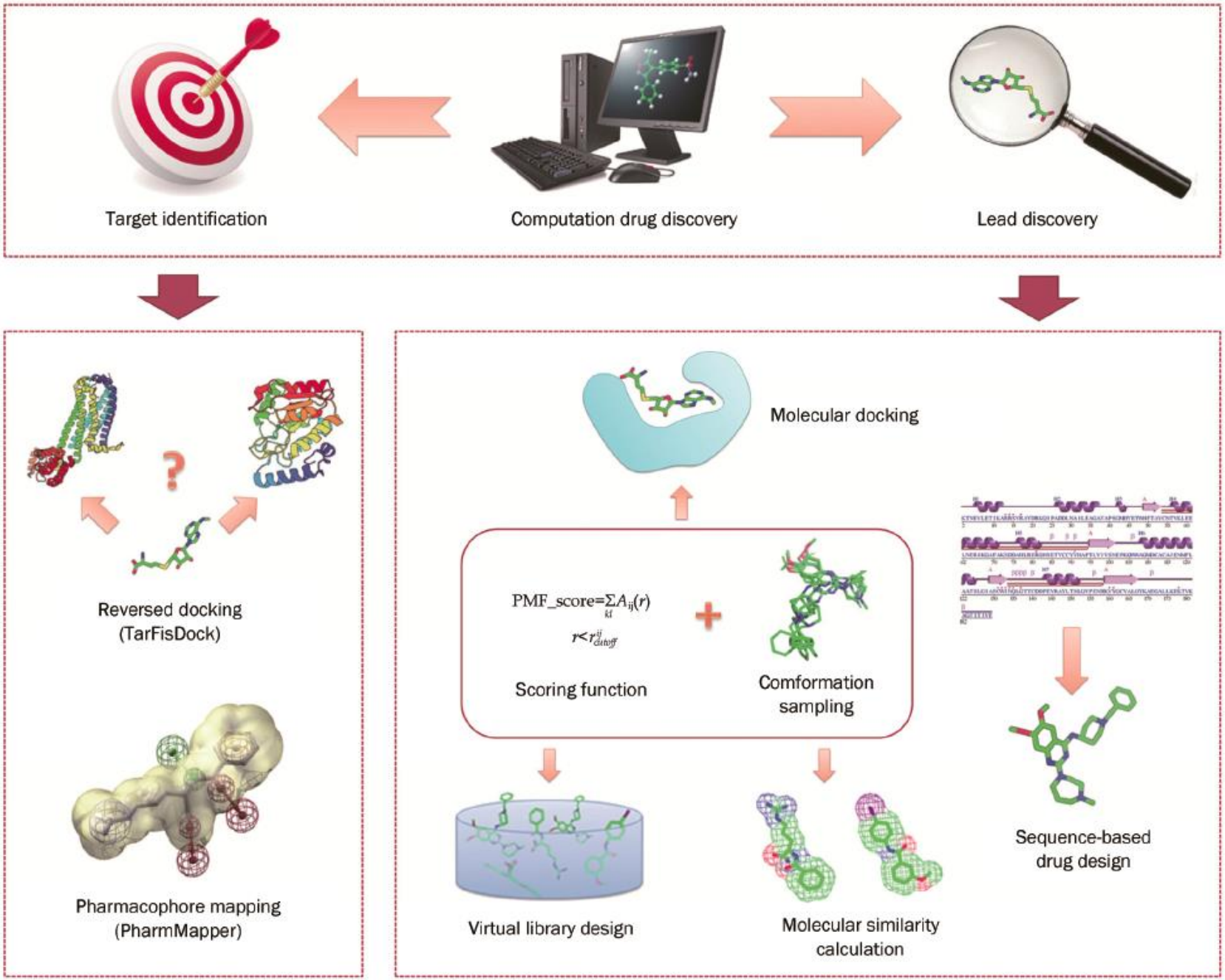


SBDD – Hit to lead phase



Drug Discovery Today

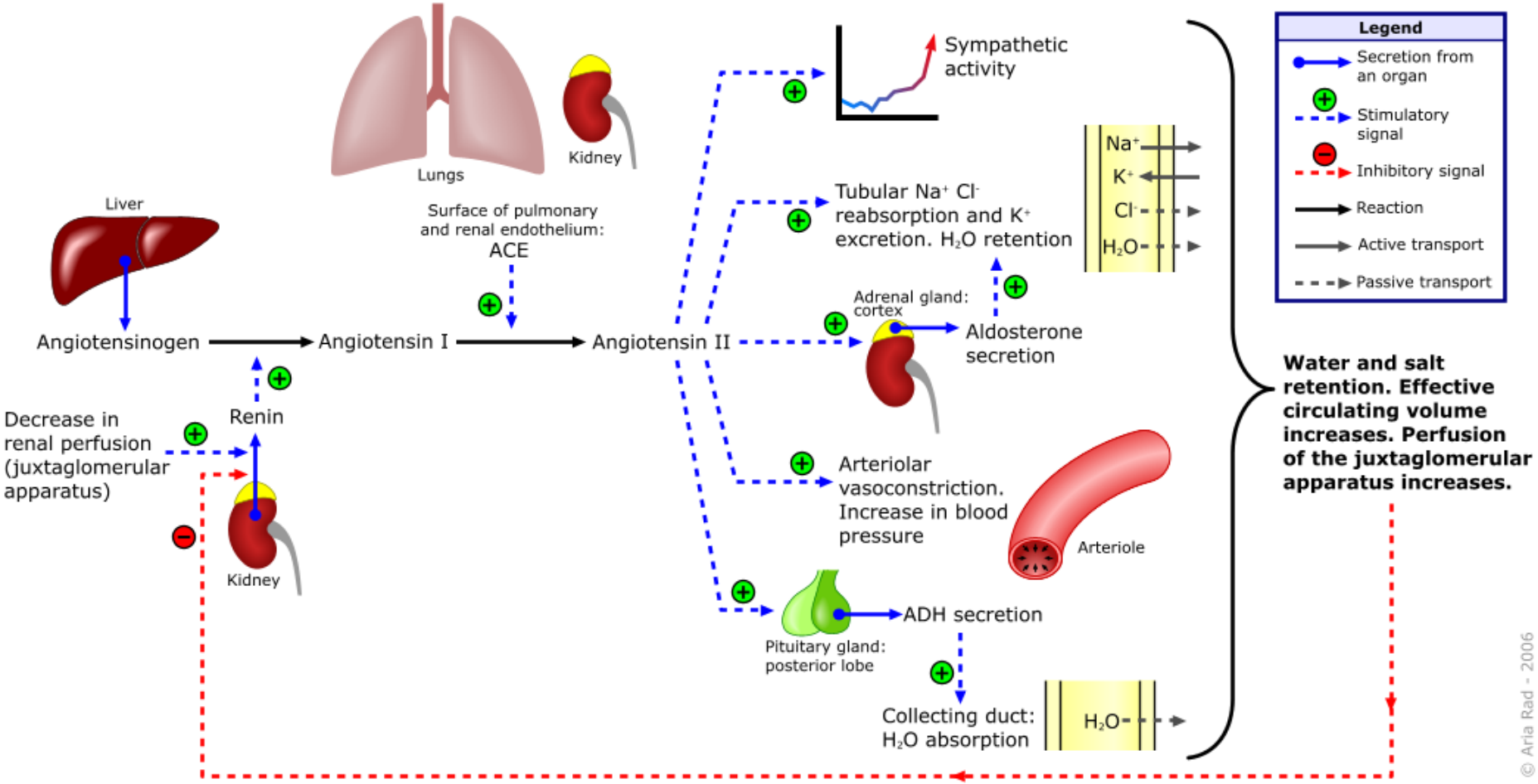

Disease-related genomics
Target identification and validation
Lead discovery and optimization
Preclinical tests
Clinical trails
• Bioinformatics
• Reverse docking
• Pharmacophore mapping
• Protein structure prediction
• Target druggability
• Chemical probe design
• Molecular docking
• Pharmacophore modeling
• De novo design
• Virtual library design
• QSAR (2D, 3D, or 4D)
• Sequence-based method
• In silico ADMET prediction
• Physiologically-based pharmacokinetic (PBPK) simulations


Target identification
Computation drug discovery
Lead discovery
Reversed docking
(TarFisDock)
Pharmacophore mapping
(PharmMapper)
Molecular docking
PMF_score=$\sum_{ij} A_{ij}(r)$
$r<r_{cutoff}^{ij}$
Scoring function
Comformation
sampling
Virtual library design
Molecular similarity
calculation
Sequence-based
drug design

Transição para o SBDD: desenvolvimento de inibidores da ACE

- O octapéptido Angiotensina II promove um aumento da tensão arterial
- Ferreira e Vane isolam um péptido do veneno da jararaca com capacidade de inbir a ACE
- Ondetti e Cushman reconhecem a similaride estrutural entre a ACE e a Carboxipeptidase A
- A estrutura da Carboxipeptidase A, bem conhecida na altura, serve de modelo à ACE
- O ácido benzil succínico é um inibidor potente da Carboxipeptidase A
- Os aminoacil-substituintes do ácido succínico revelaram-se inbididores potentes da ACE!

Angiotensin II : mechanisms of action

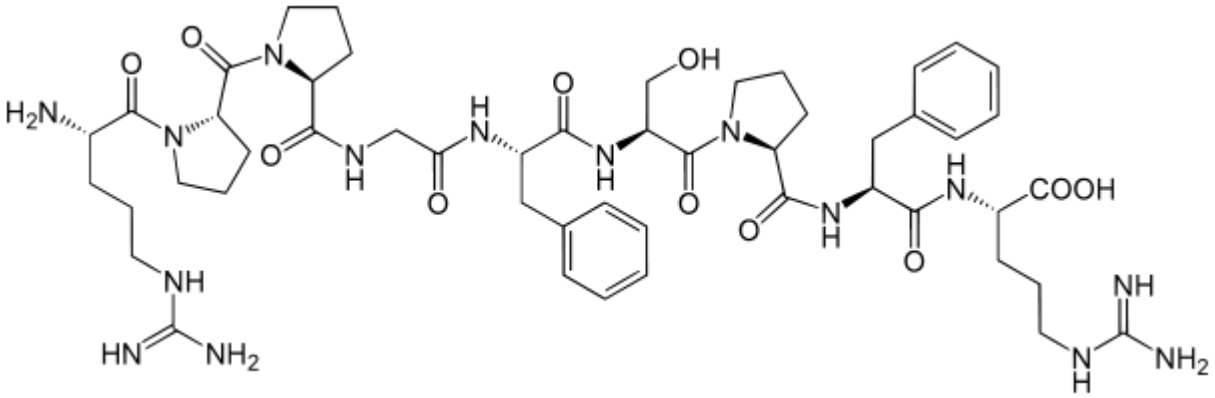


ACE also degrades the **blood-pressure-lowering** nonapeptide **bradykinin**

Angiotensina I Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

ACE (angiotensin-converting enzyme) → His-Leu

Angiotensina II Asp-Arg-Val-Tyr-Ile-His-Pro-Phe



Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

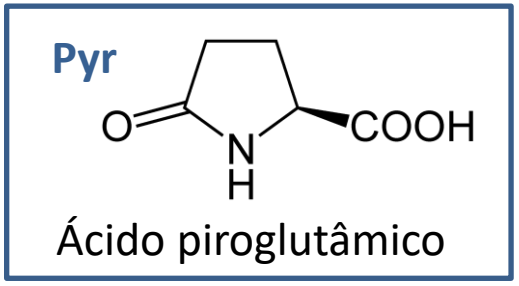
Bradicinina

Descoberta do teprótido

Em 1965 Sérgio Ferreira e Joseph Vane isolam do veneno da cobra brasileira jararaca uma mistura peptídica capaz de prolongar o efeito anti-hipertensivo da bradicinina por inibição da sua degradação. Desta mistura foi isolado o péptido **teprótido**, de fórmula:

Pyr-Trp–Pro–Arg–Pro–Gln–Ile–Pro–Pro

Foi posteriormente demonstrado que este péptido inibe também a formação de Angiotensina II a partir da Angiotensina I.
Miguel Ondetti, da farmacêutica Squibbs, sintetizou este péptido, o qual se verificou ser um potente inibidor da ACE (Ki = 100 nM), tanto em animais como em humanos, embora não pudesse ser usado como fármaco - os péptidos não são geralmente assimiláveis por via oral.



Ácido piroglutâmico

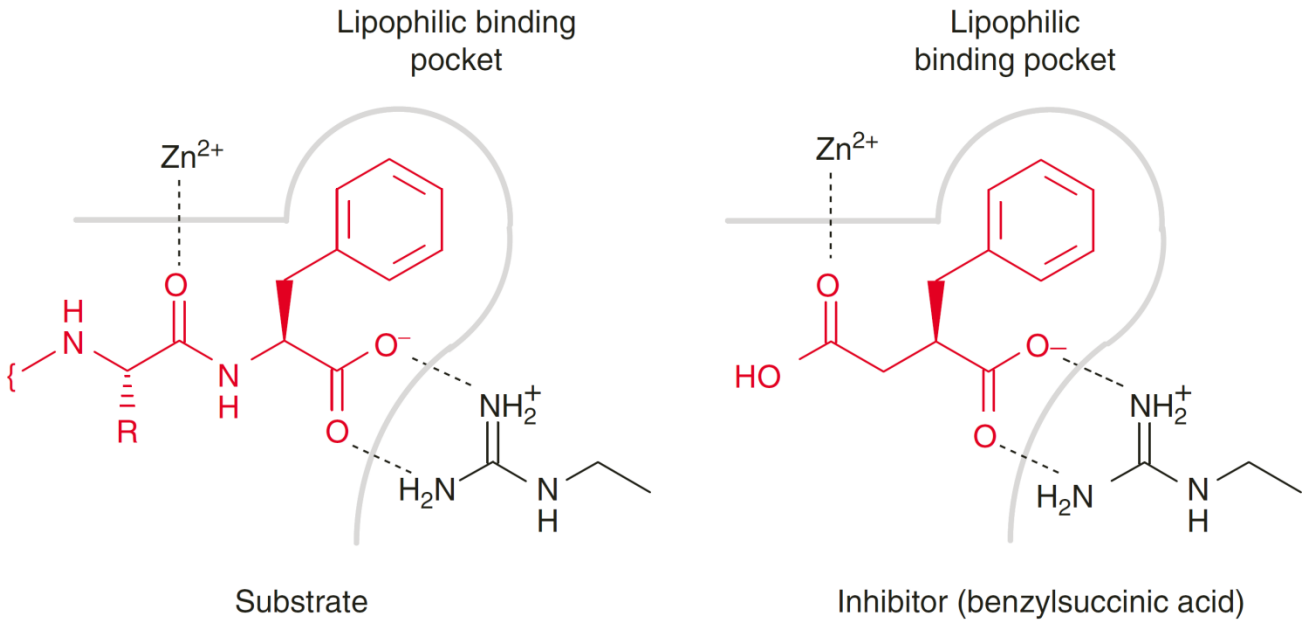

Jararaca (*Bothrops jararaca)*

Ferreira SH (1965*) Br J Pharmacol Chemother* 24(1):163–169

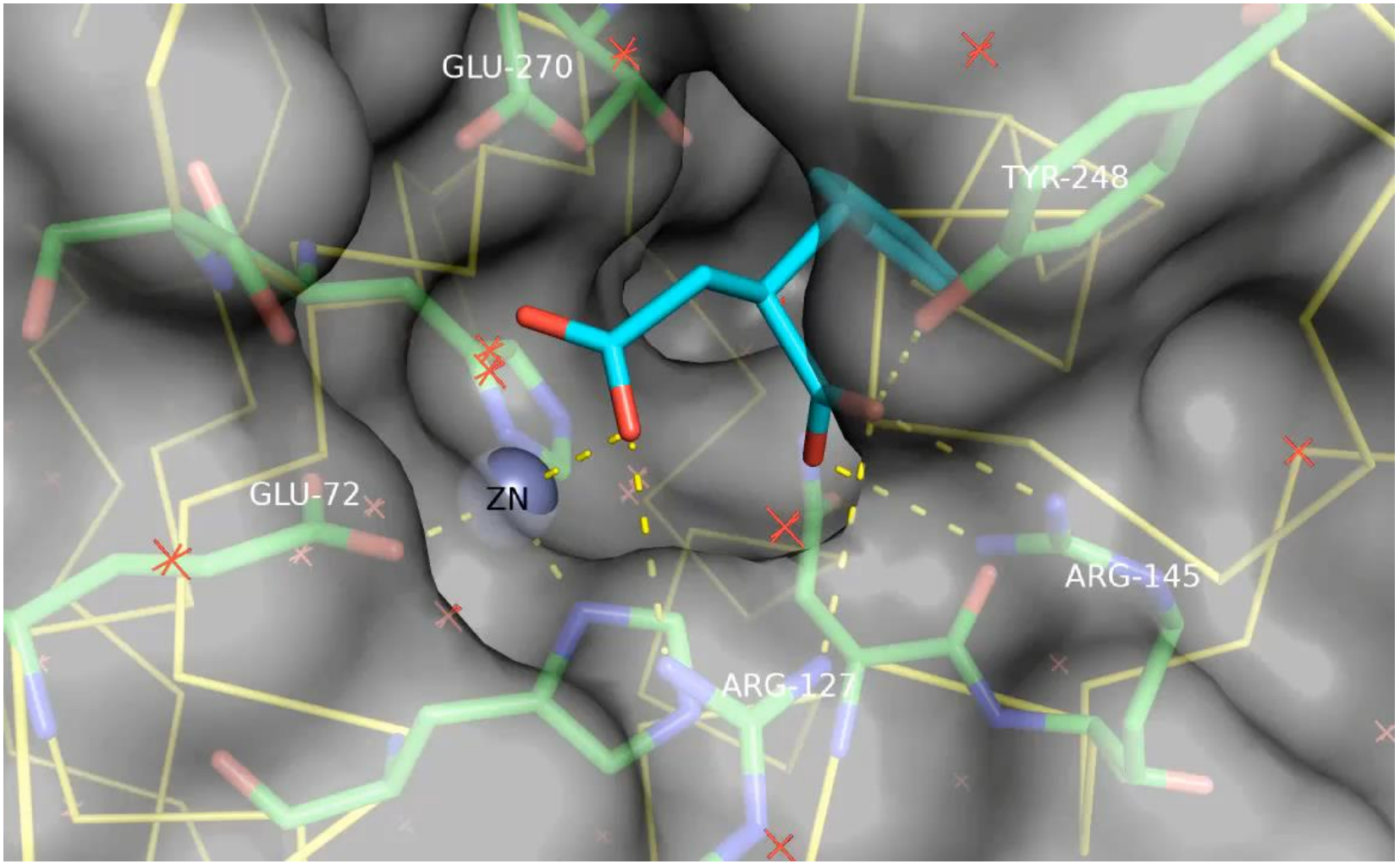
Carboxypeptidase A *versus ACE*

Nos anos 70 Miguel Ondetti e David Cushman (Squibbs) reconhecem a similarirdade de mecanismo entre a ACE e *carboxypeptidase A*, uma enzima que remove aminoácidos C-terminais de uma cadeia polipeptídica.

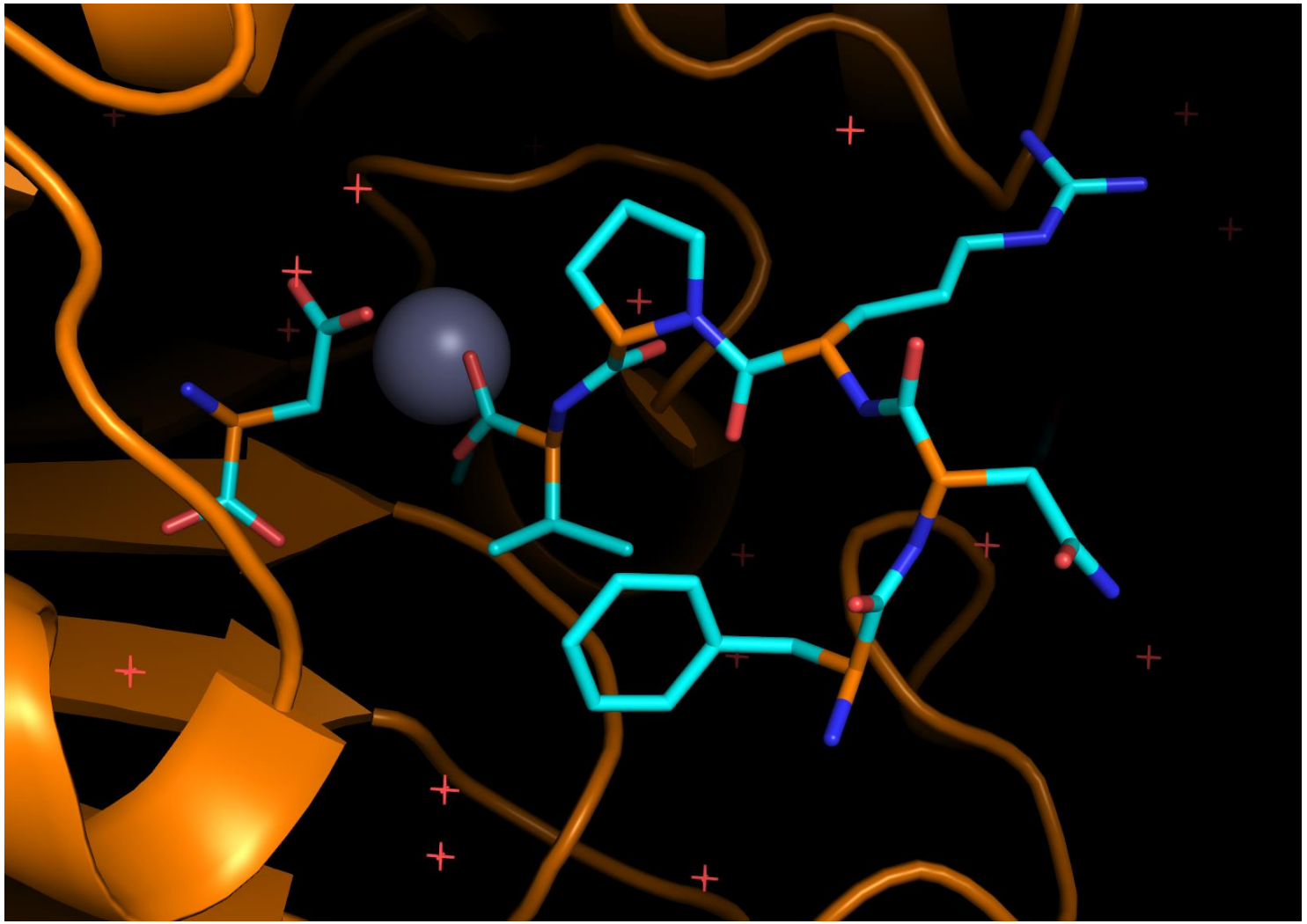
O ácido benzil-succínico é um potente inibidor da Carboxypeptidase A, funcionando com um análogo da região C-terminal do substrato.

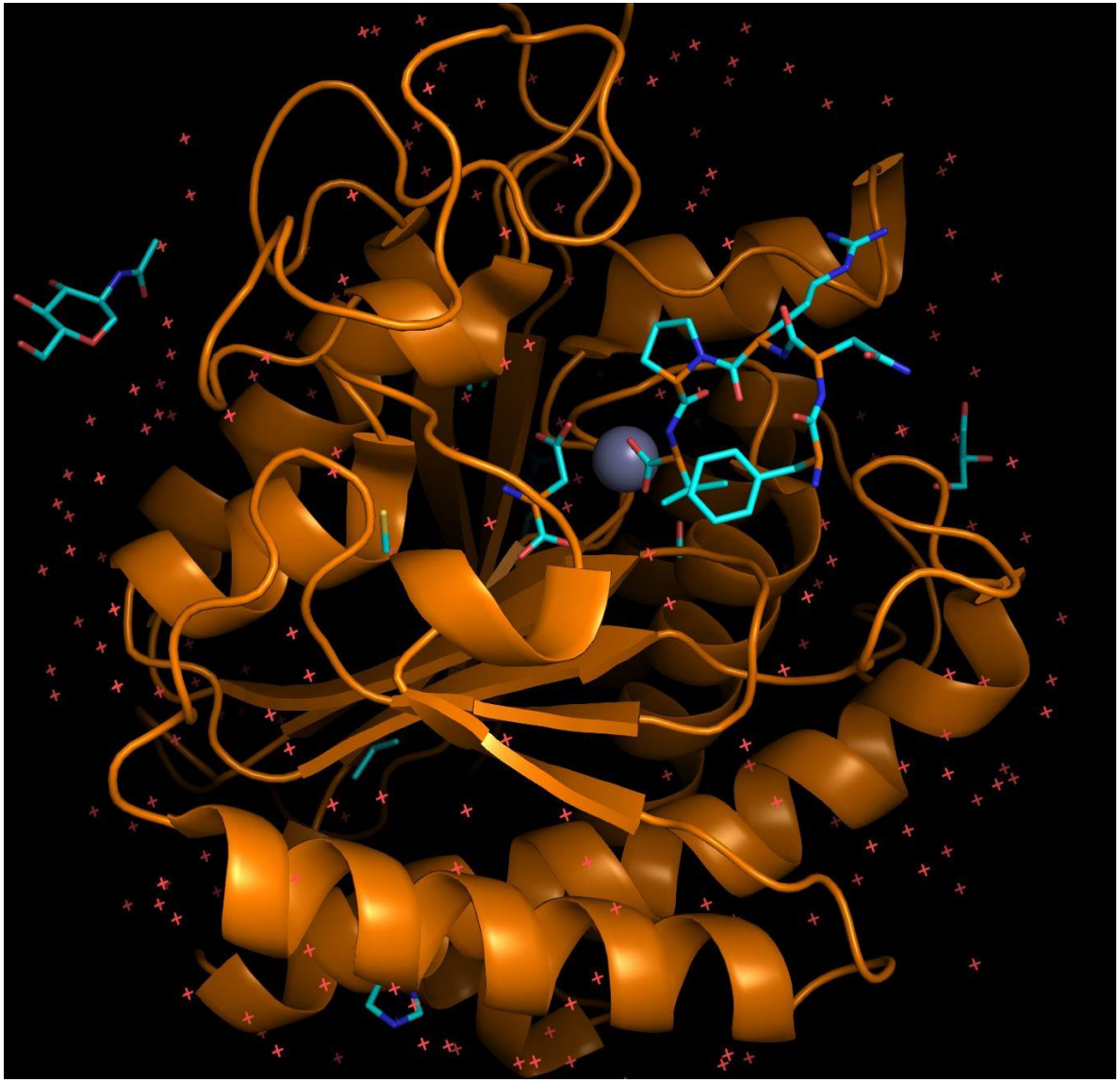


Carboxypeptidase A em complexo com o inibidor benzil succinato

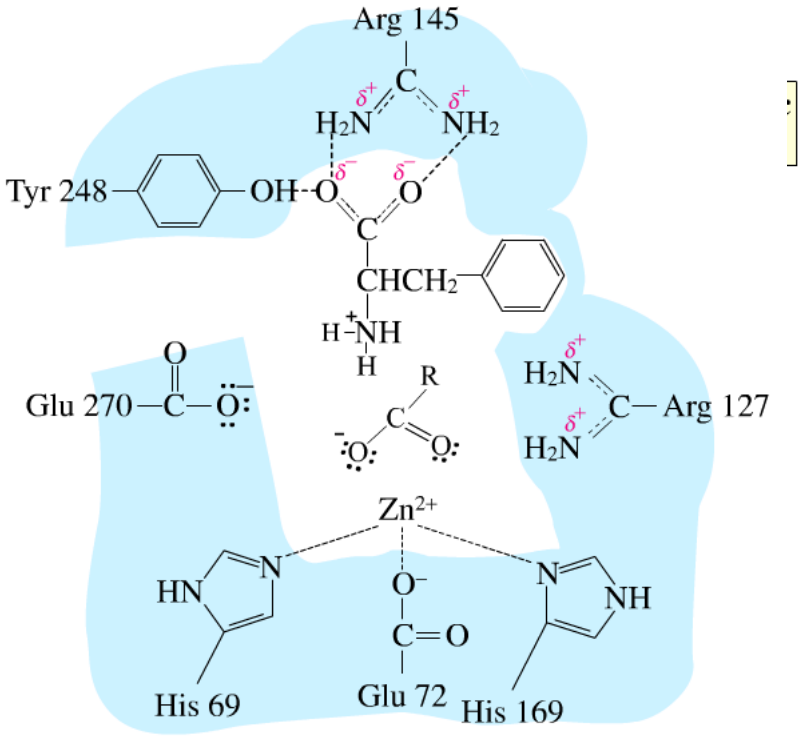


Péptido clivado no centro activo da Carboxipeptidase A

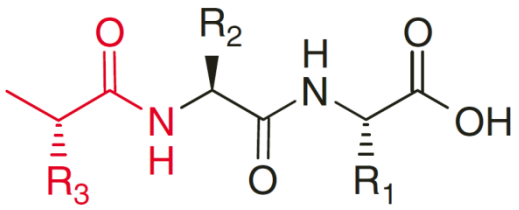




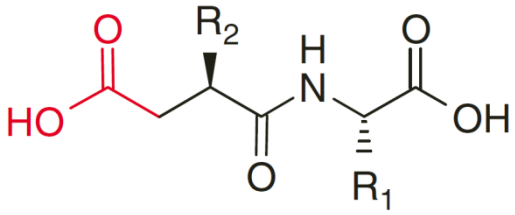
Mecanismo catalítico da Carboxipeptidase A



Dado que a ACE remove os *dois* resíduos C-terminais, Ondetti e Cushman investigaram a possibilidade usar derivados amino-substituídos do ácido succínico como inibidores desta enzima.

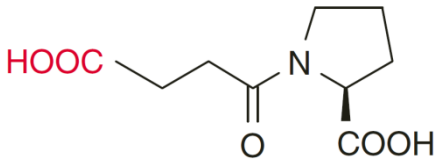


Substrate



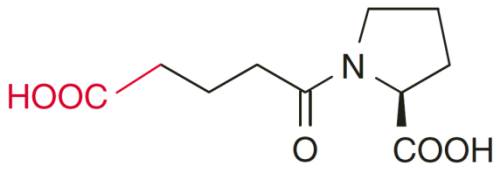
Inhibitor

Ala–Pro (Ki = 230 mM)

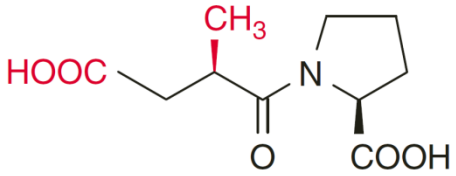


Succinil-L-prolina (IC_{50} = 330 μM)

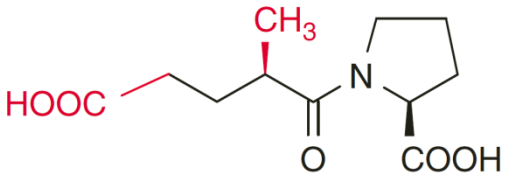
Optimização do lead



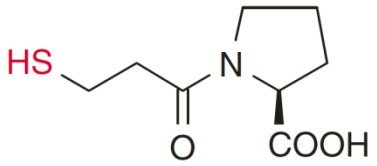
25.9 IC_{50} = 70 μM



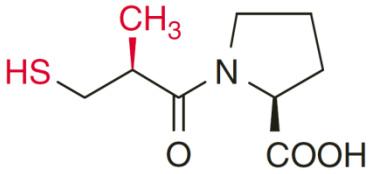
25.10 IC_{50} = 22 μM



25.11 IC_{50} = 4.9 μM

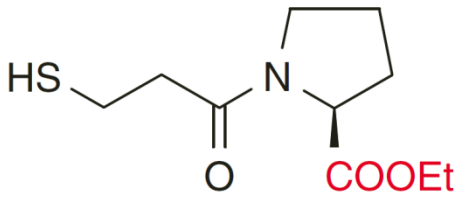


25.12 IC_{50} = 200 nM
K_i = 12 nM

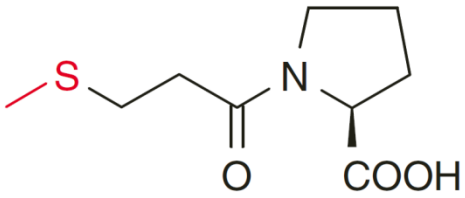


25.13 Captopril

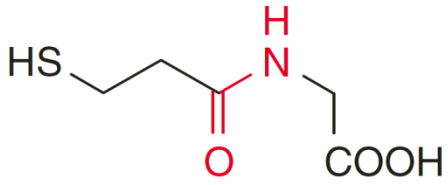
IC_{50} = 23 nM
K_i = 1.7 nM



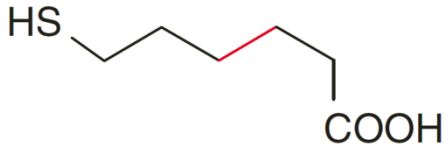
25.14 IC_{50} = 17 μM



25.15 IC_{50} = 4300 μM

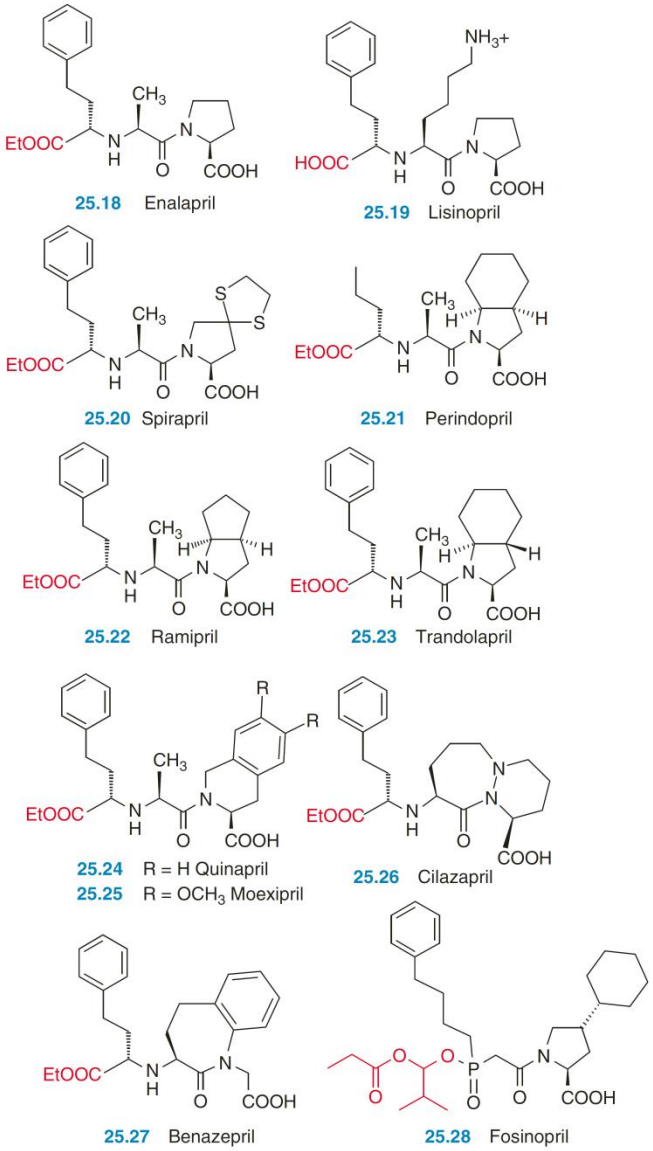


25.16 IC_{50} = 2.8 μM



25.17 IC_{50} = 1100 μM

Estudos com estes compostos indicaram a necessidade do grupo tiol, grupo carboxílico livres e da presença do anel de prolina.



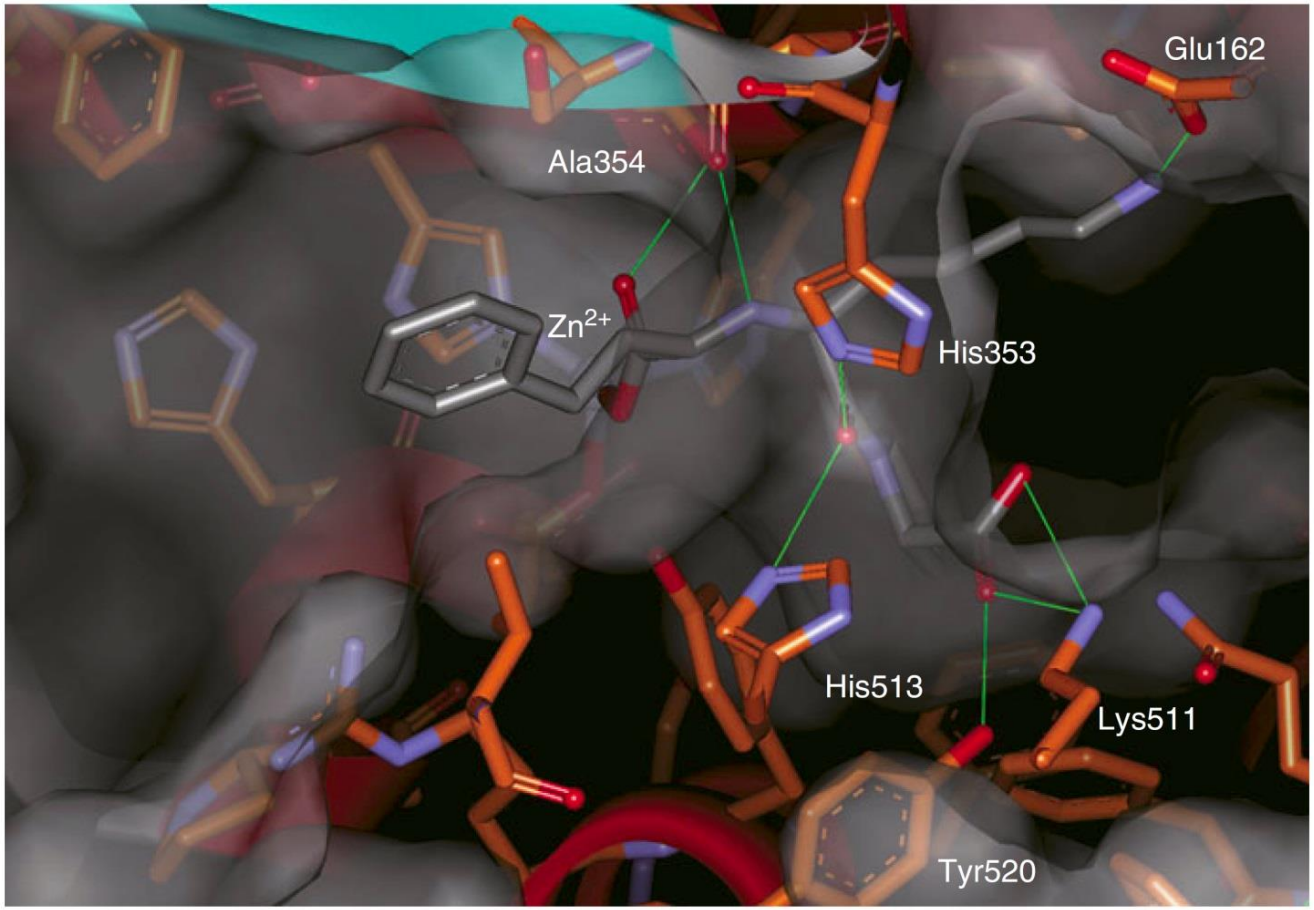
Outros inibidores da ACE desenvolvidos posteriormente.

The studies described above exemplify the great heuristic value of an active-site model in the design of inhibitors, even when such a model is a hypothetical one. Only when suitable information on substrate specificity and mechanism of action of an enzyme is available can one make a reasonable working hypothesis with regard to complementary functionality needed in an inhibitor.

-- David Cushman

- A ACE humana apresenta duas formas: t-ACE (testicular) e s-ACE (somática)
- A estrutura da t-ACE em complexo com o inibidor lisinopril foi resolvida em 2003 por Edward Sturrock e colaboradores
- A s-ACE é constituída por dois domínios catalíticos (N e C) os quais apresentam alta similaridade estrutural de sequência, entre si e com a t-ACE
- As estruturas dos domínios N e C foram resolvidas posteriormente, revelando importantes diferenças estruturais com impacto na especificidade e função fisiológica dos dois domínios
- O domínio C desempenha um papel mais importante na regulação da tensão arterial enquanto o domínio N participa na regulação das células estaminais hematopoéticas
- Os inibidores da ACE sem especificidade de domínio acarretam um maior risco de efeitos colaterais indesejados (possivelmente liga dados à inibição da degradação da bradicinina).
- A clivagem da Angiotensina I ocorre preferencialmente no domínio C, enquanto a bradicinina é degradada em ambos os domínios
- A inibição dirigida ao domínio C permite inibir a formação de angiotensina II, mantendo uma atividade residual de degradação da bradicinina no domínio N.
- A busca de inibidores específicos para domínio é um dos aspetos mais importantes no desenvolvimento de novos inibidores da ACE

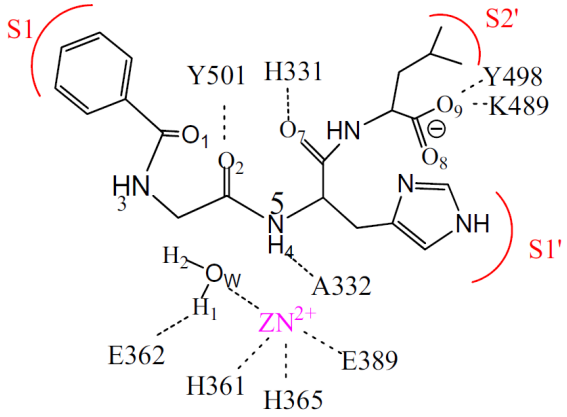
Em 2003 o grupo de Edward Sturrock determinou a estrutura cristalográfica da t-ACE em complexo com o lisinopril. Posteriormente foi resolvida a estrutura da s-ACE, mostrando a presença de dois domínios com actividade catalítica (domínio N-terminal e domínio C-terminal) e sequências diferentes (~50% de identidade).



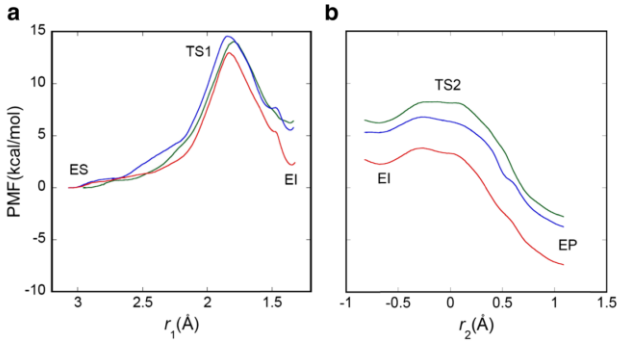
t-ACE em complexo com o **lisinopril**

Natesh *et al.* (2003) Nature **421**:551

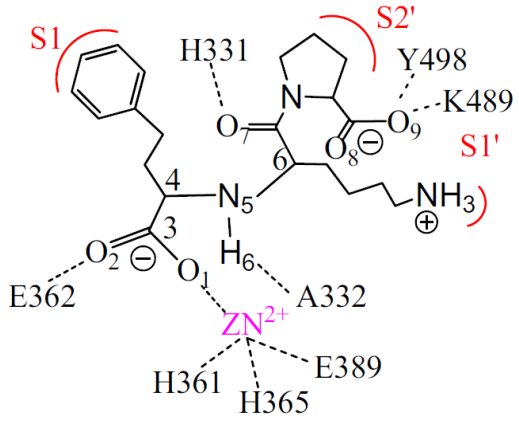
Mecanismo catalítico da ACE (hipotético)



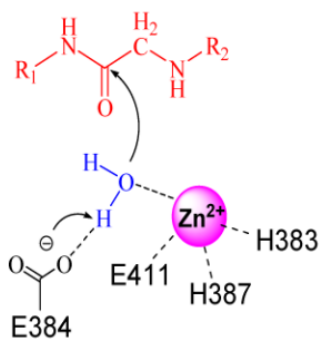
Hip-His-Leu



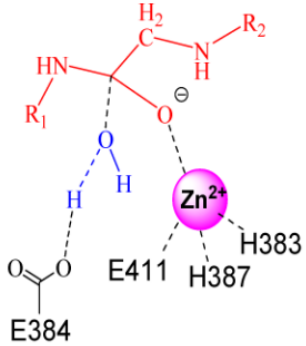
Perfil de energia da reacção



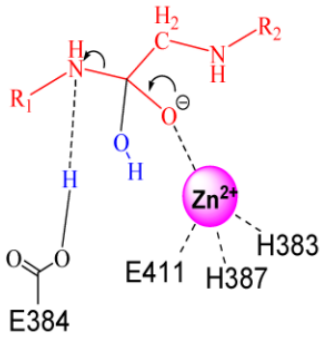
Lisinopril



ES



TS1



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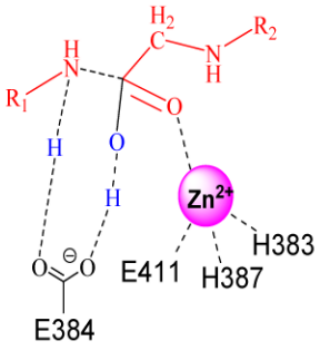
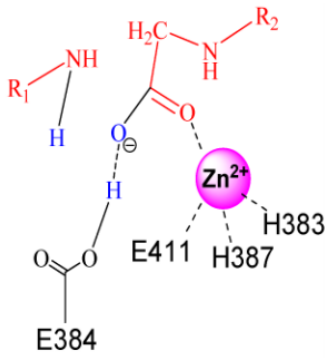


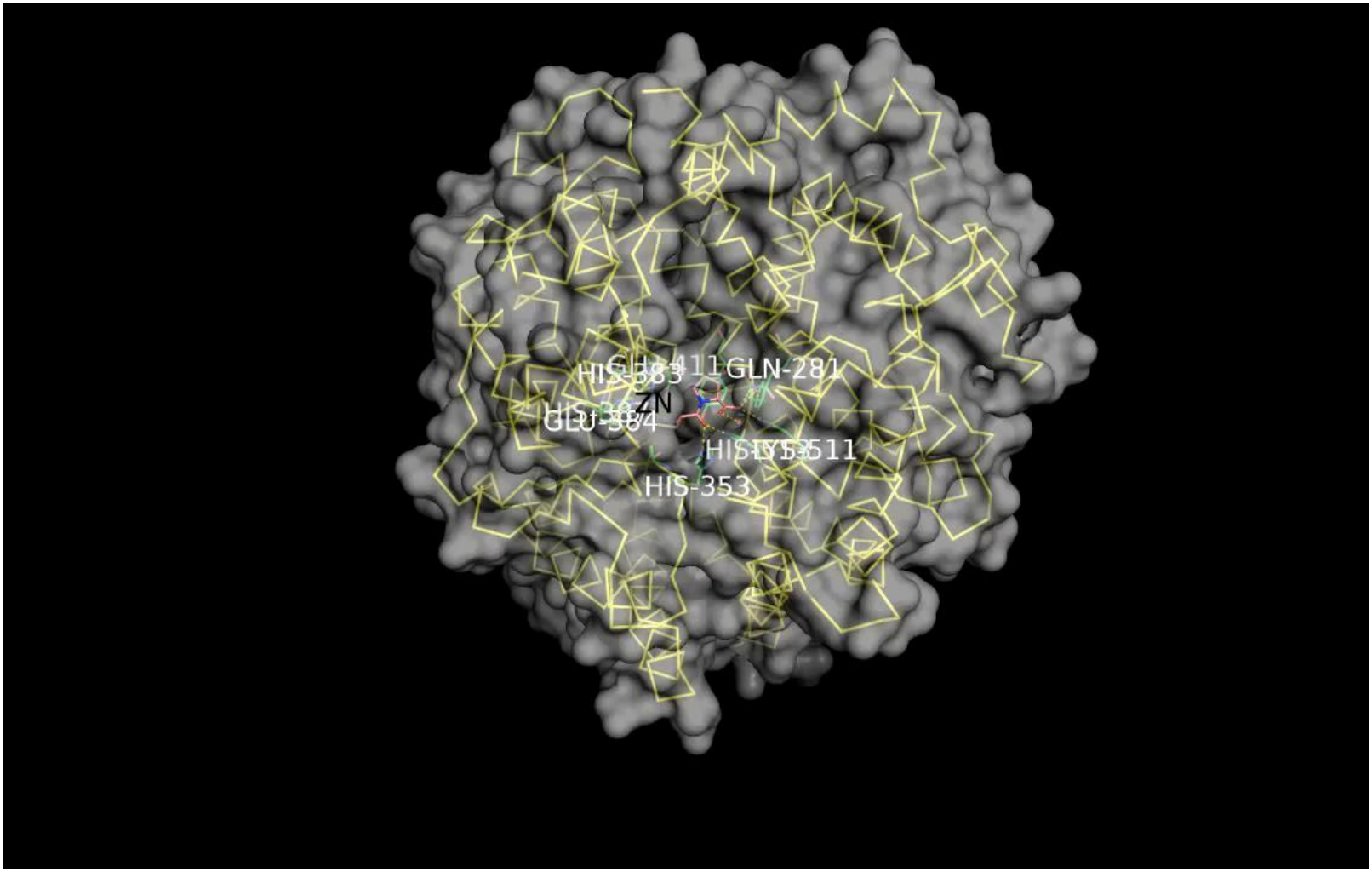
TS2



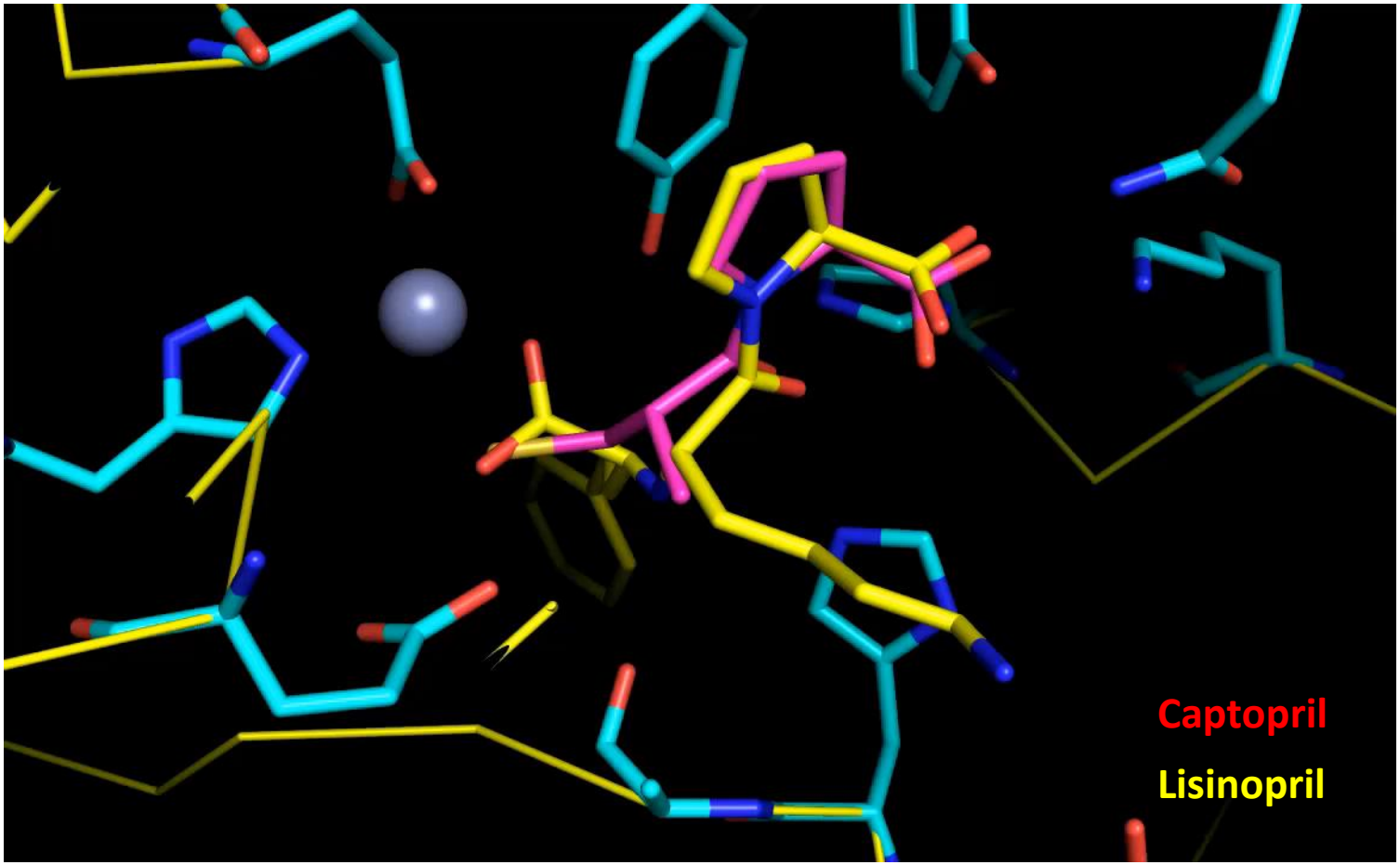
EP

Mu *et al.* (2016) *J. Mol. Model.* **22**:132

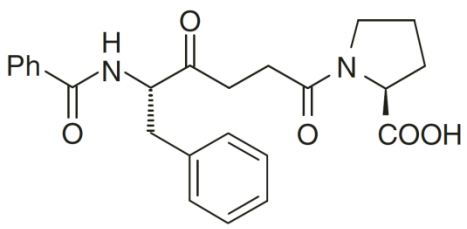
t-ACE humana em complexp com o captopril



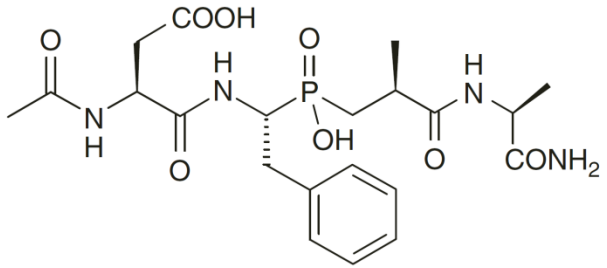
Comparação do captopril e lisinopril no centro active da t-ACE



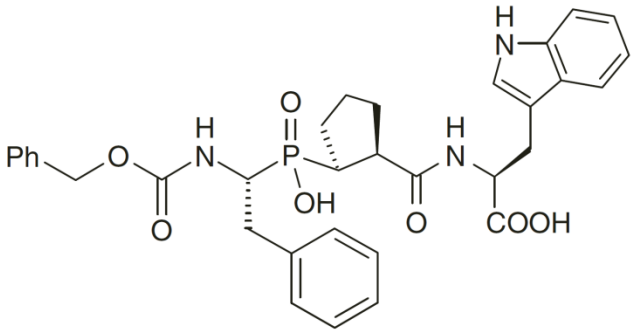
Inibidores específicos para os domínios N e C da ACE



25.29 Keto-ACE



25.30 RXP407

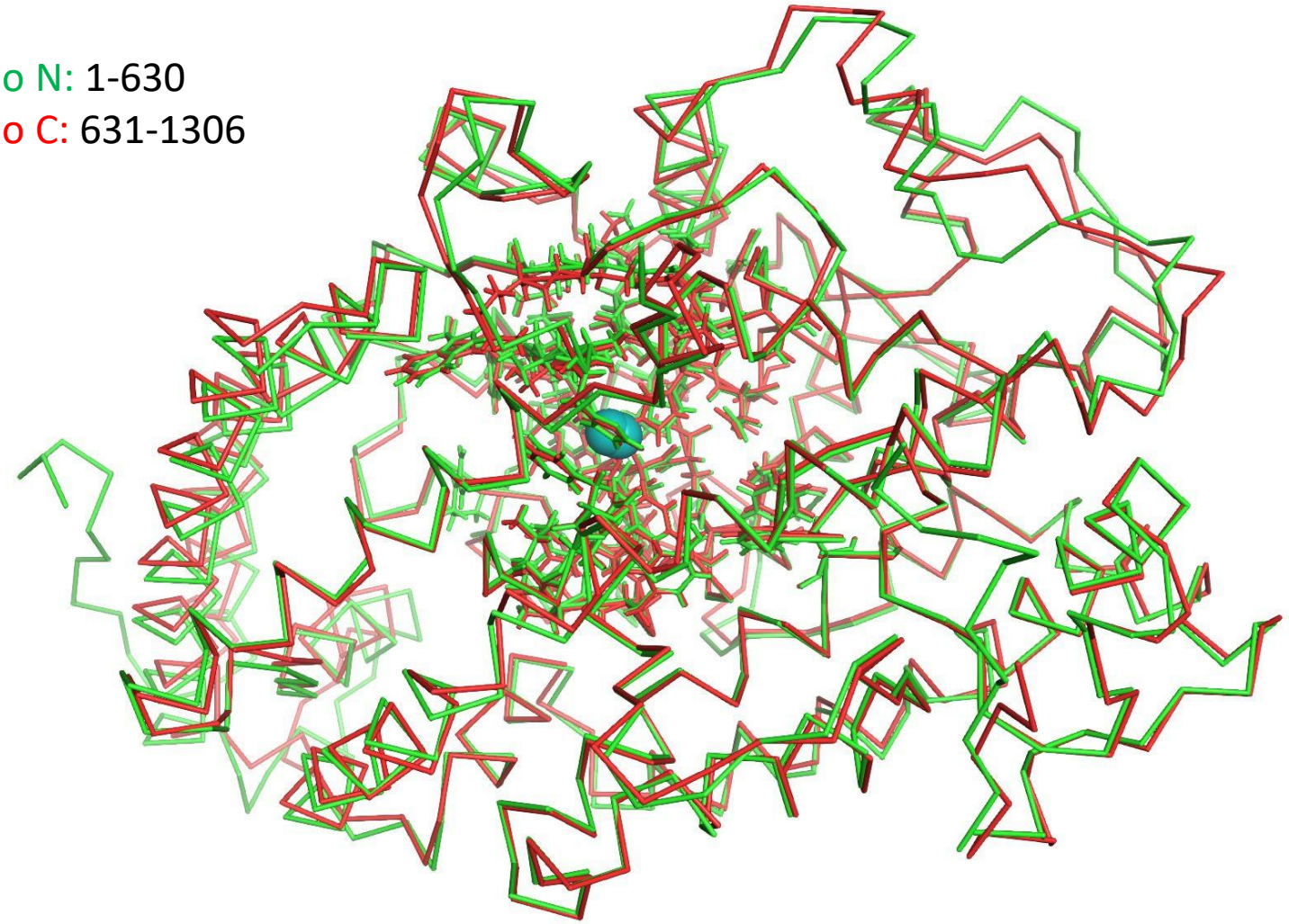


25.31 RXPA380

Compound	N-domain inhibition (nM)	C-domain inhibition (nM)
RXP A380 **25.31**	10,000	3.0
Captopril **25.13**[a]	8.9	14.0
Enalapril **25.18**[b]	26.0	6.3
RXP407 **25.30**	2.0	2,500
Lisinopril **25.19**[b]	44.0	2.4
Keto-ACE **25.29**	15,000	40.0

Comparação dos domínios C e N da s-ACE humana

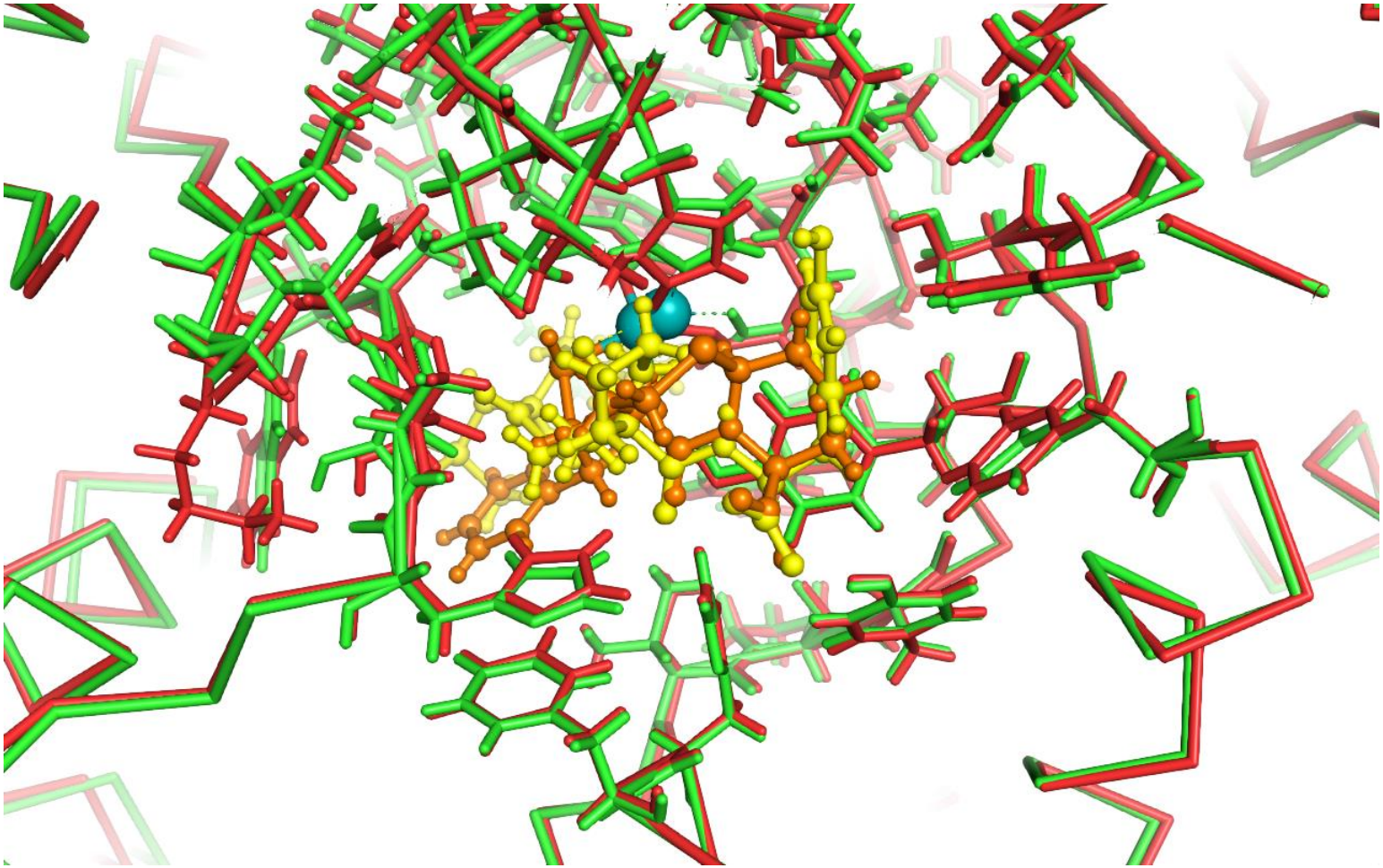
Domínio N: 1-630
Domínio C: 631-1306



PDBs: 6H5W, 6F9V

Percentage de identidade de sequência entre os domínios: 53%

s-ACE humana



Sampatrilat

Omapatrilat

Domínio N: 1-630

Domínio C: 631-1306

Progresso no desenvolvimento de novos inbidores da ACE

- Geração de novas moléculas sintéticas com especificidade de domínio
- Pesquisa de produtos naturais com menor toxicidade e efeitos colaterais
- Pesquisa e identificação em fontes naturais de péptidos com catividade anti-ACE
- Pesquisa e desenho de polissacáridos inibidores da ACE
- Geração de bibliotecas de péptidos *in silico* para docking e virtual screening

Geração e pesquisa de uma biblioteca de tripétptidos inibidores da ACE

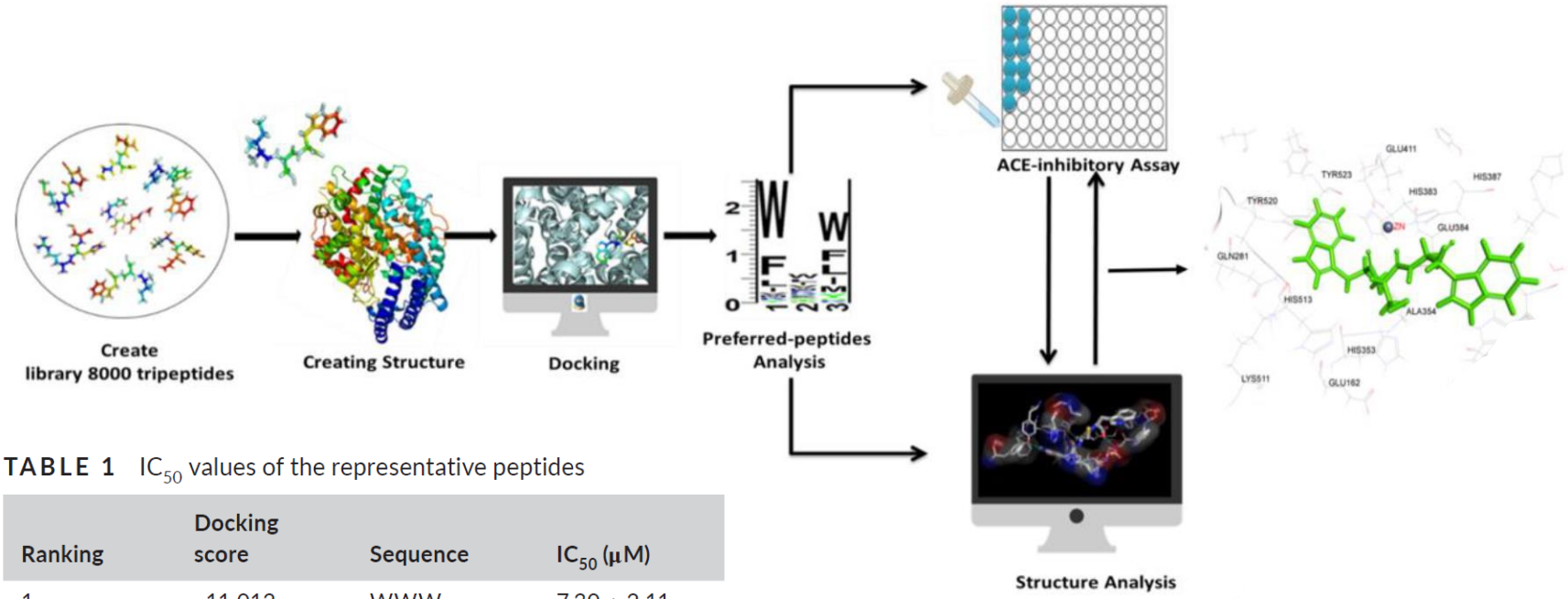


TABLE 1 IC_{50} values of the representative peptides

Ranking	Docking score	Sequence	IC_{50} (μM)
1	−11.012	WWW	7.30 ± 2.11
2	−10.777	KYY	20.46 ± 4.05
3	−10.616	WRF	21.84 ± 2.50
4	−10.613	WRY	5.86 ± 0.73
5	−10.603	WQW	11.83 ± 1.79
7,999	−1.778	DGG	>5,000
8,000	−1.604	GGG	>5,000
		Captopril	0.037 ± 0.008

Note: Data are expressed as mean ± *SD*.

Panyayai et al. (2021) Comput Biol Chem. 77:207-213

Chen et al. (2021) Food Sci Nutr. 9:2943–2953