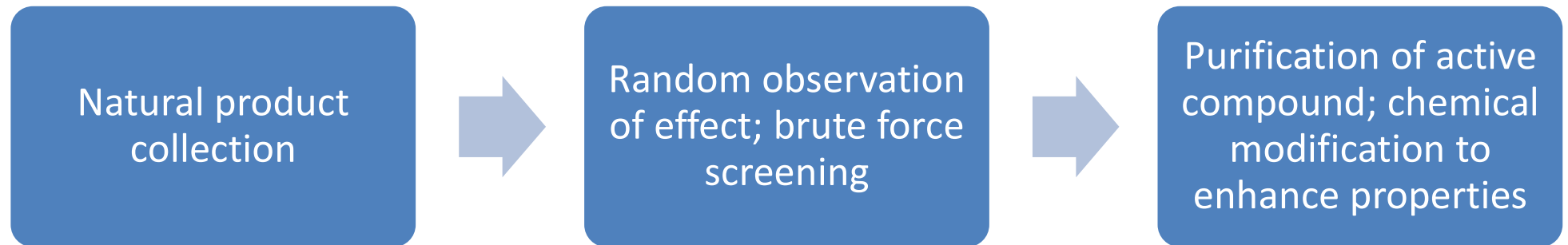


Desenho de fármacos: racional *versus* “irracional”

- 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

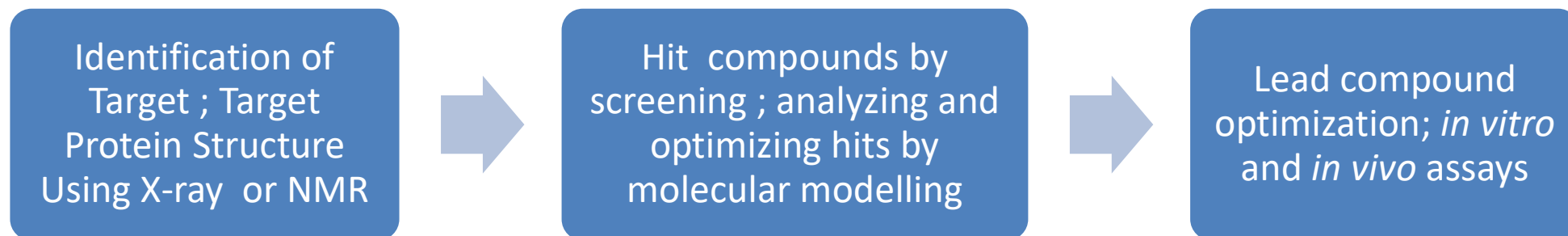
Irrational drug design approach



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 known salicylate plant extracts. Bayer marketed the substance in 1999 under the name Aspirin and began selling it worldwide.

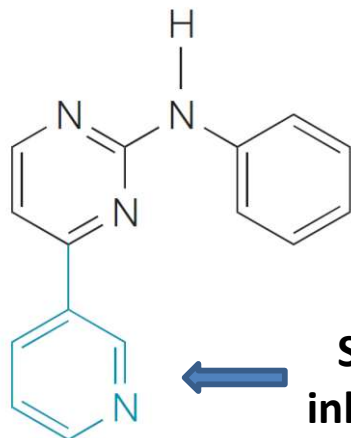
Rational drug design approach



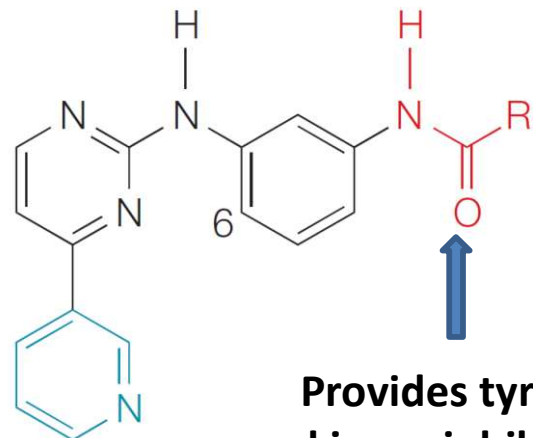
Example: The drug *Gleevec* for the treatment of Chronic Myelogenous Leukemia (CML) was found by screening a library of compounds against Protein Kinase C active. Compounds that showed activity against PKC were chemical modified based on computational modelling studies and X-ray structures of their interaction 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)

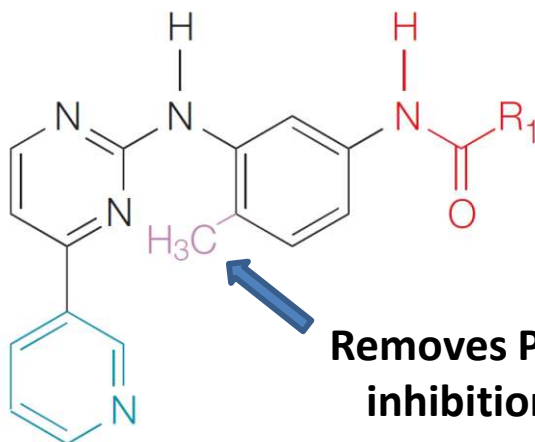
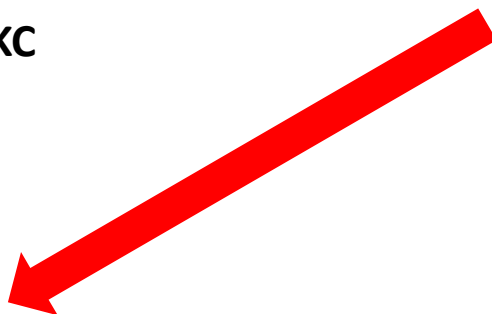
Found on a screen against PKC activity:



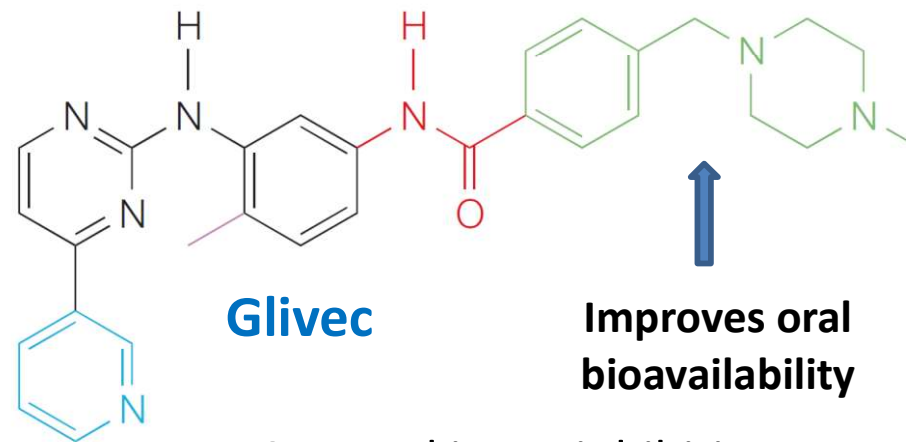
Strong inhibition against PKC



Provides tyrosine kinase inhibition



Removes PKC inhibition



Glivec

Improves oral bioavailability

BCR-ABL kinase inhibition

Chronic Myelogenous Leucemia

Structure *versus* Ligand-Based Drug Design

	Known Ligands	Unknown Ligands
Know protein structure	Structure-based drug design (SBDD) Protein modelling Docking-guided Chemical optimization	<i>De novo</i> design
Unknown protein structure	Ligand-based drug design (LBDD) <i>1 or more ligands</i> <ul style="list-style-type: none">• Similarity searching <i>Several ligands</i> <ul style="list-style-type: none">• Pharmacophore searching <i>Many ligands (20+)</i> <ul style="list-style-type: none">• Quantitative Structure-Activity Relationships (QSAR)	No rational approach Need experimental data of some sort Can apply ADMET filters

ADMET: absorption, distribution, metabolism, excretion, toxicity

Desenho de fármacos baseado em estrutura

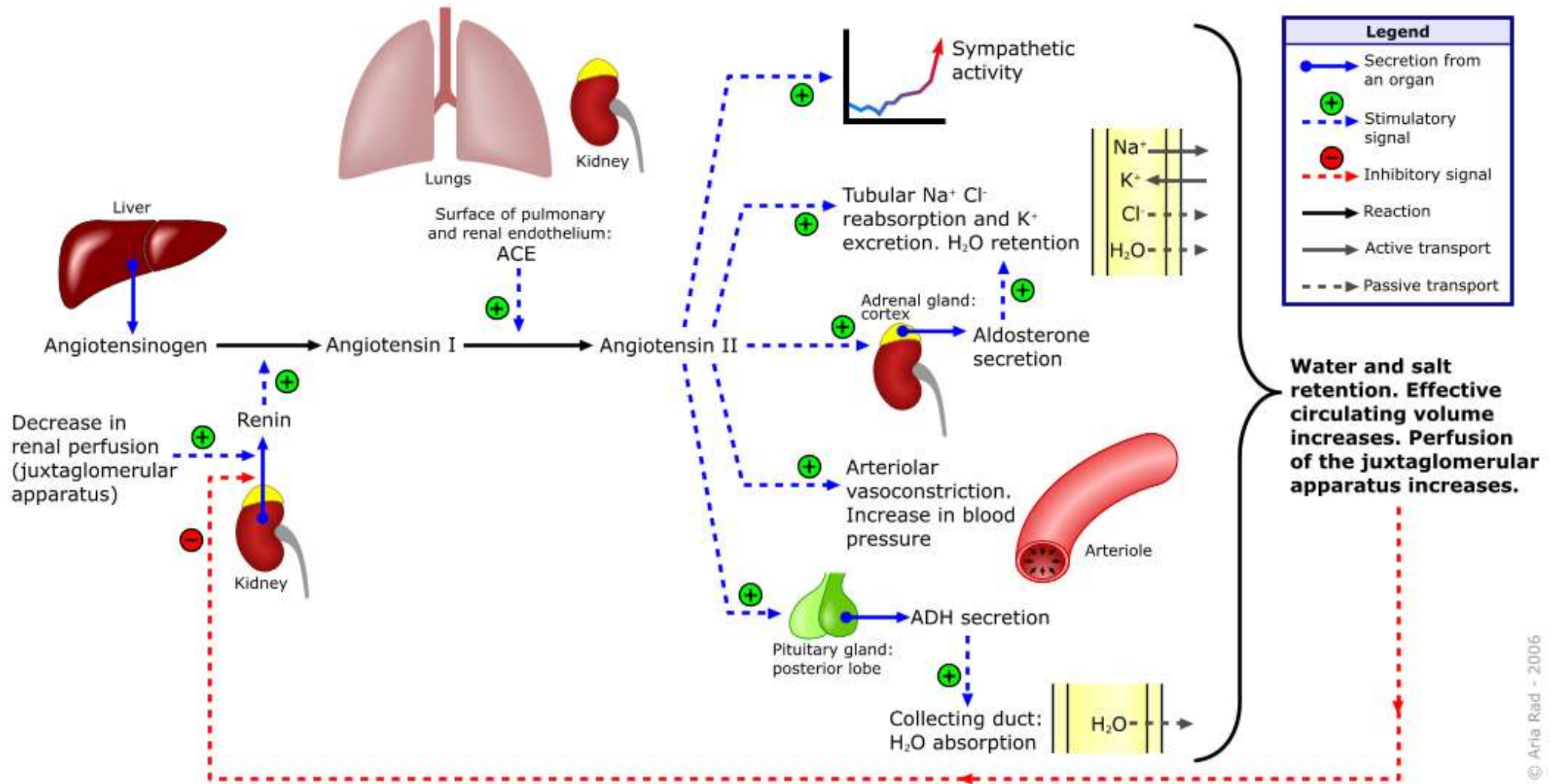
- O SBDD tornou-se possível com o desenvolvimento de uma vasta biblioteca de estruturas de receptores e enzimas
- Neste tipo de abordagem a forma e características electrónicas do centro activo são consideradas desde o início
- As estruturas cristalográficas da proteína alvo e do ligando são determinadas 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 !

Um exemplo de 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 inibir a ACE
- Ondetti e Cushman reconhecem a similaridade 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 aminoácil-substituintes do ácido succínico revelaram-se inibidores potentes da ACE!

Angiotensin II : mechanisms of action

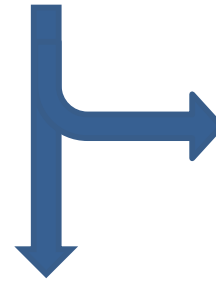


ACE also degrades the **blood-pressure-lowering** nonapeptide **bradykinin**

Angiotensina I

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

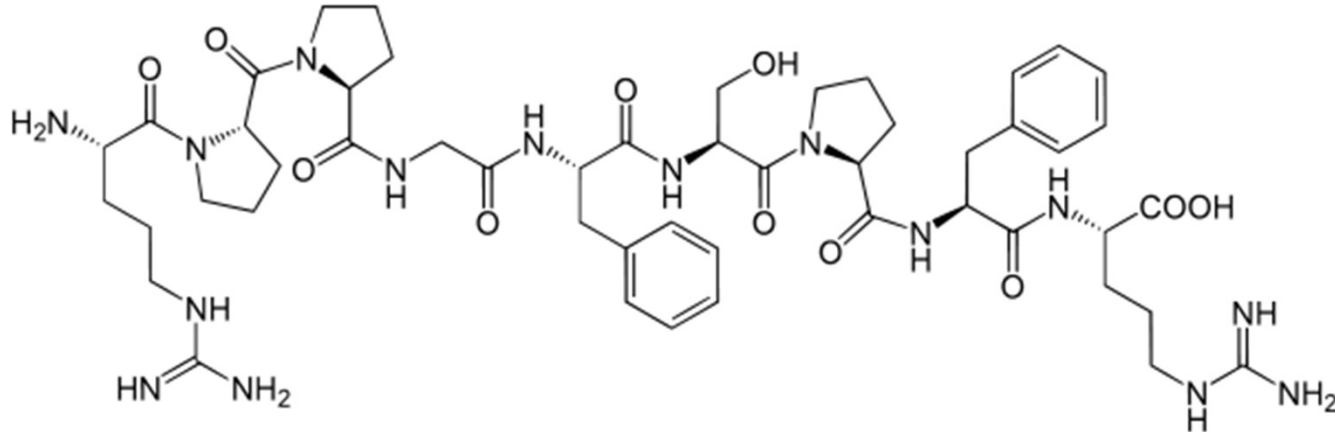
ACE (angiotensin-converting enzyme)



His-Leu

Angiotensina II

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



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

Bradicinina

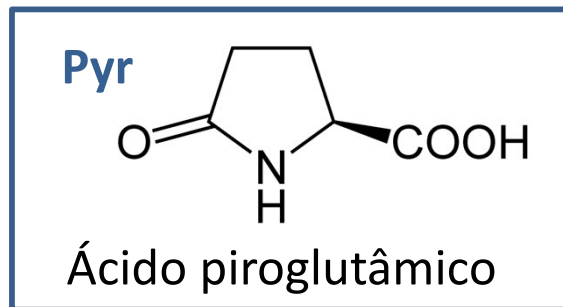
Descoberta do teprótido

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:



Foi posteriormente demonstrado que este péptido inibe também a formação de Angiotensina II a partir da Angiotensina I.

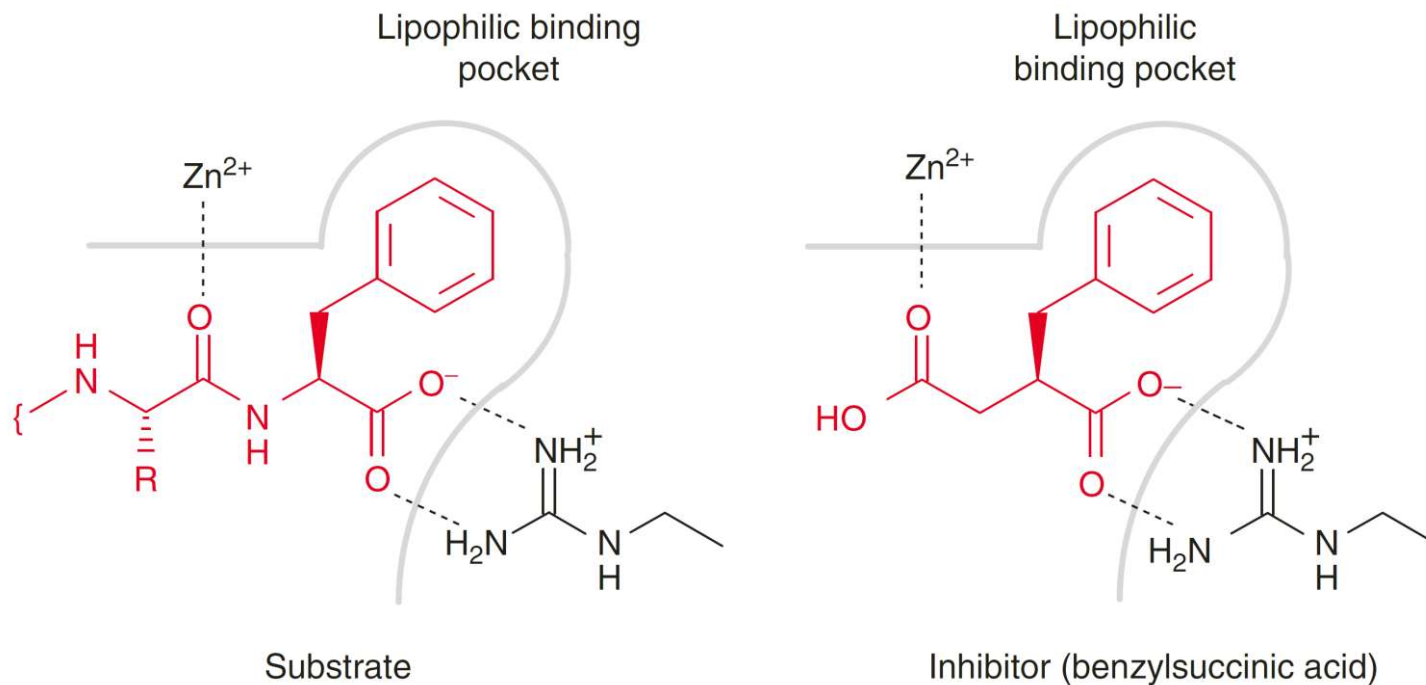
Miguel Ondetti, da farmacêutica Squibbs, sintetizou este péptido, o qual se verificou ser um potente inibidor da ACE ($K_i = 100 \text{ nM}$), tanto em animais como em humanos, embora não pudesse ser usado como fármaco - os péptidos não são geralmente assimiláveis por via oral.



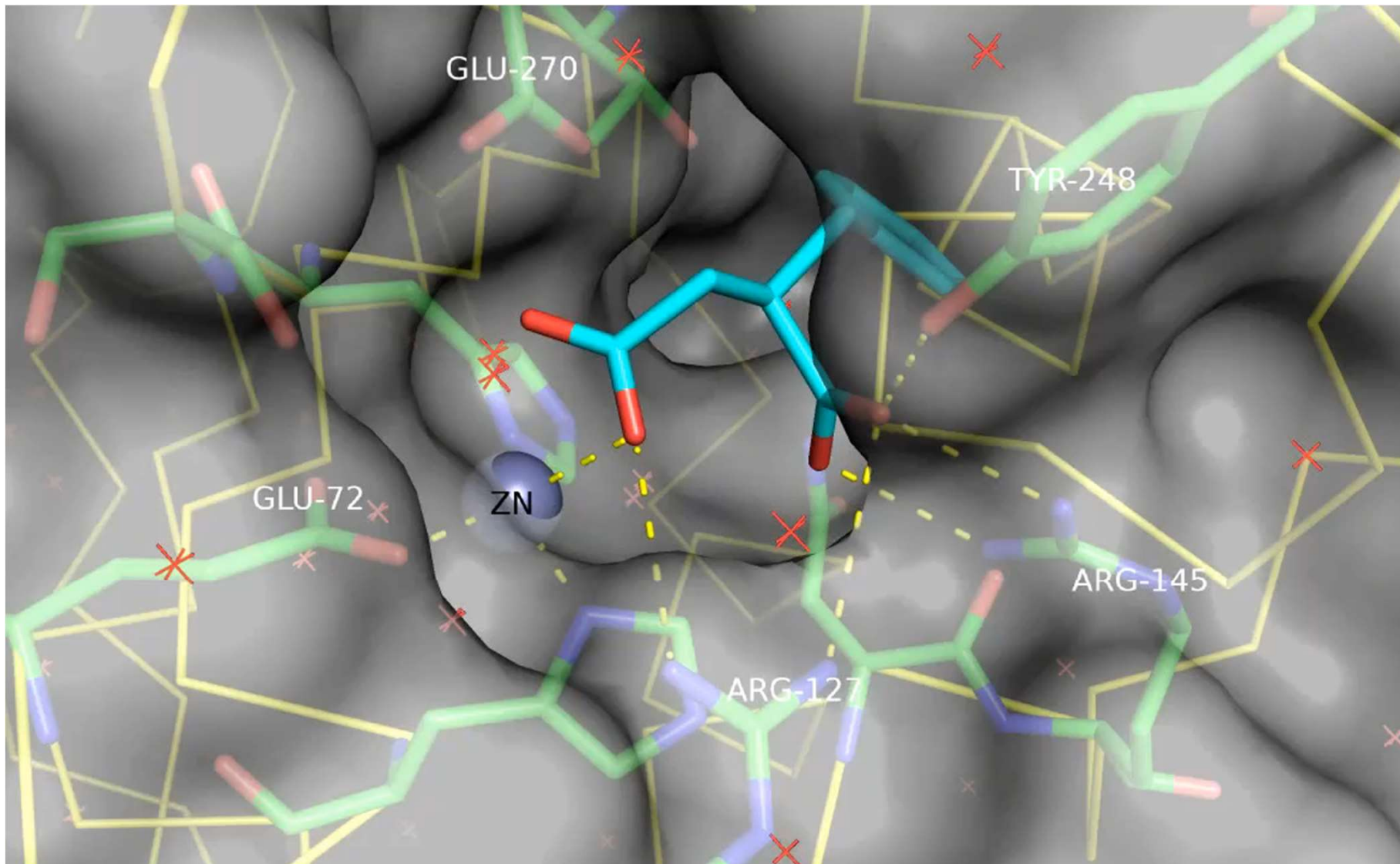
Carboxypeptidase A *versus* ACE

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

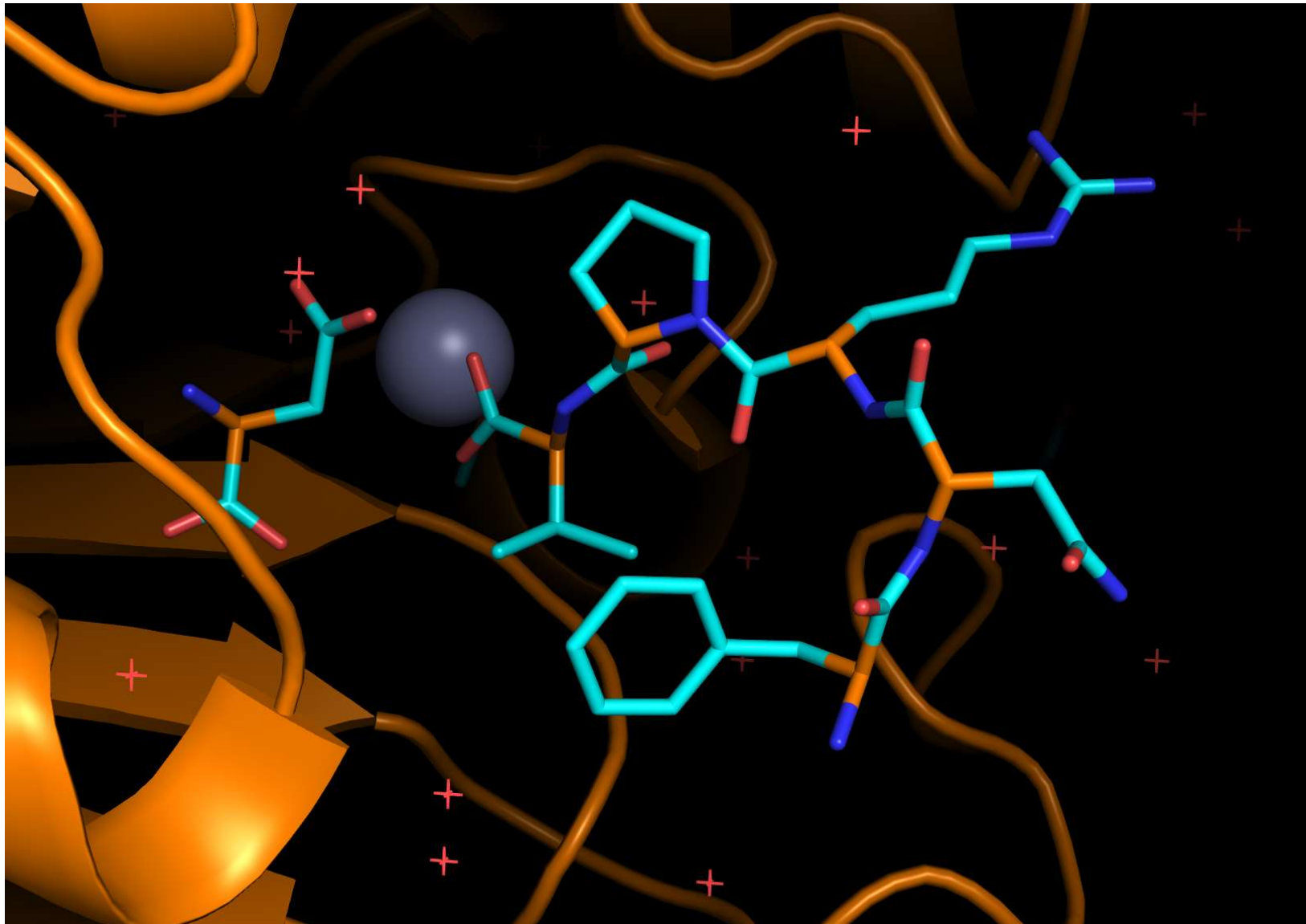
O ácido benzil-succínico é um potente inibidor da Carboxypeptidase A, funcionando com um análogo da região C-terminal do substrato.

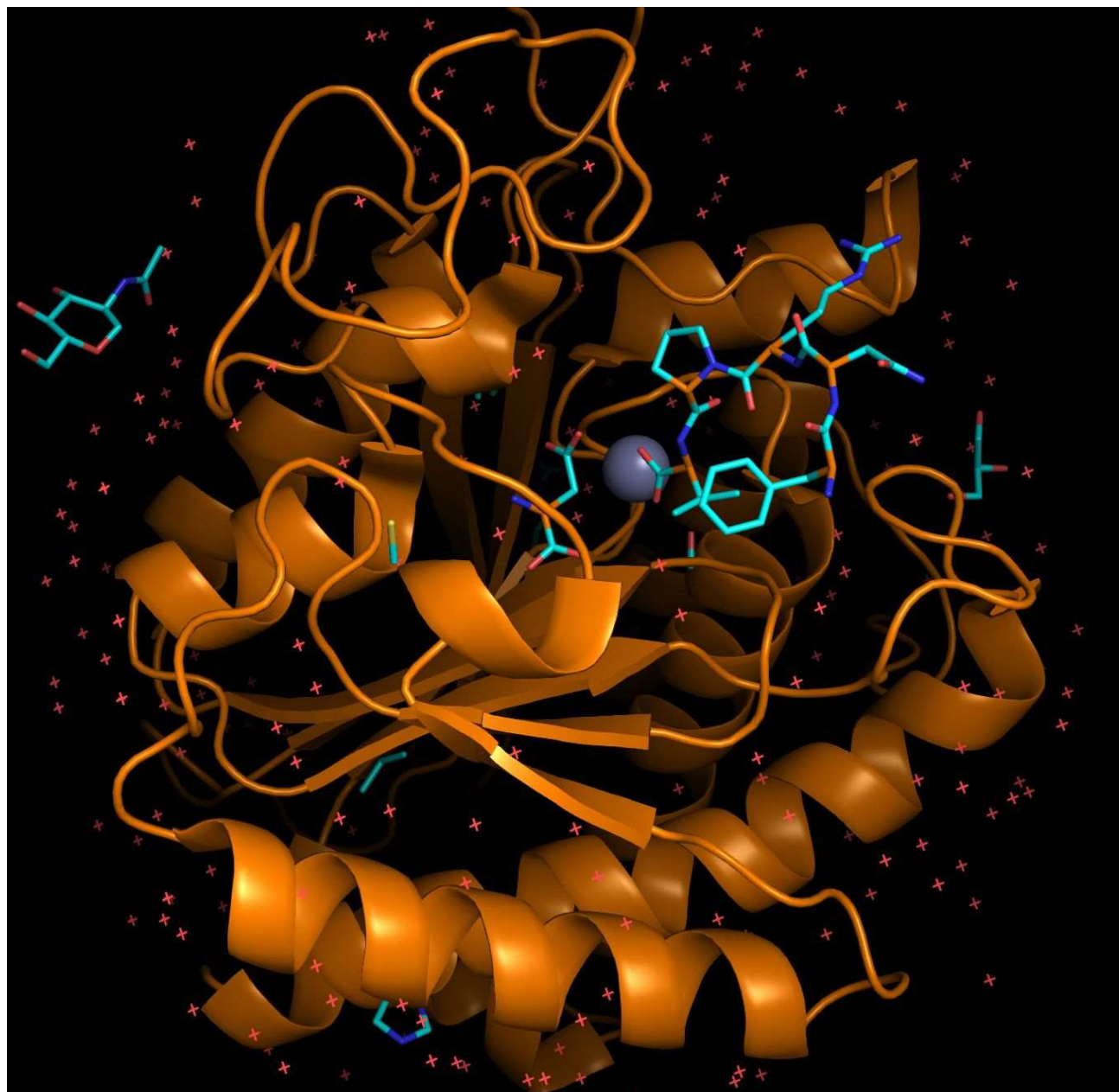


Carboxypeptidase A em complexo com o inibidor benzil succinato

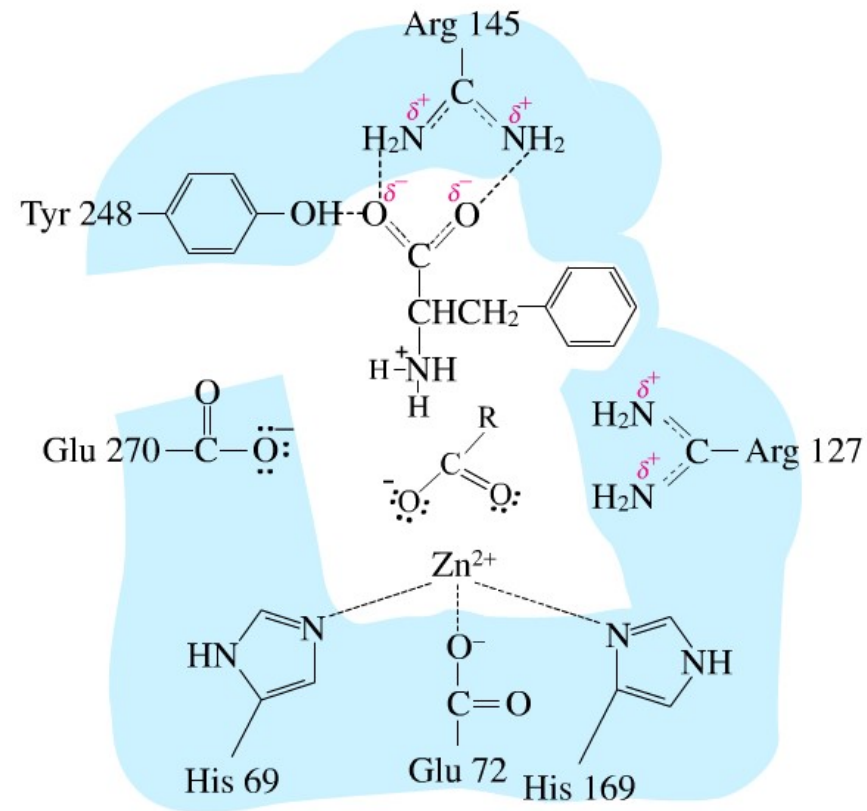


Péptido clivado no centro active da Carboxipeptidase A

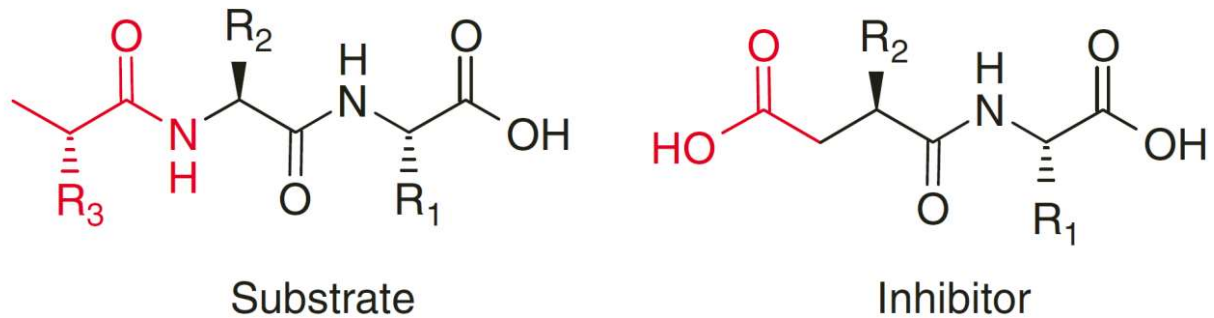




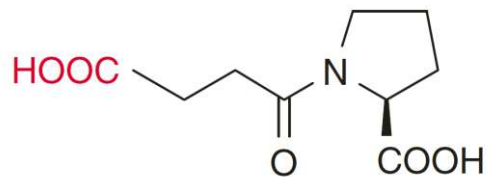
Mecanismo catalítico da Carboxipeptidase A



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.

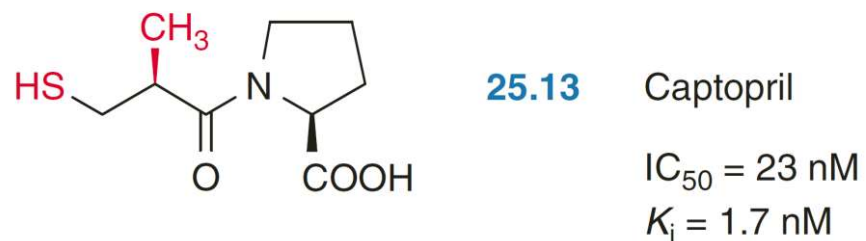
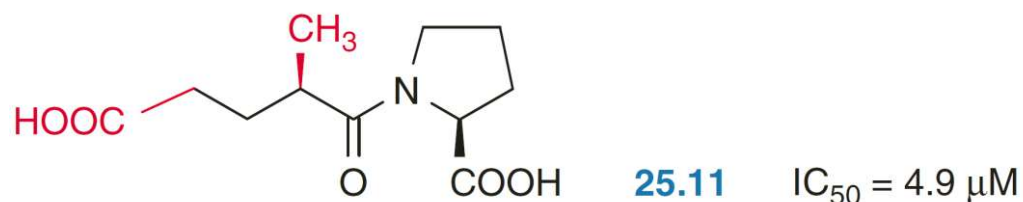
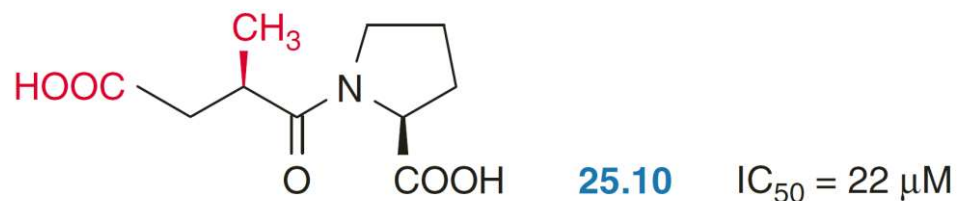
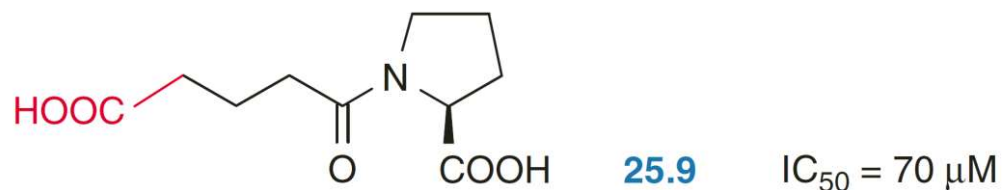


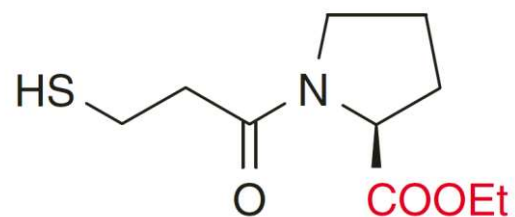
Ala-Pro (K_i = 230 mM)



Succinil-L-prolina (IC₅₀ = 330 μM)

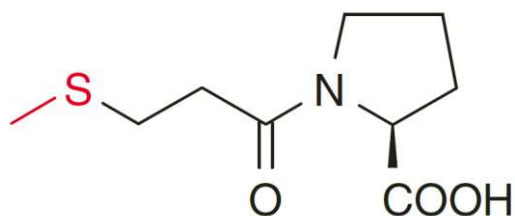
Optimização do lead





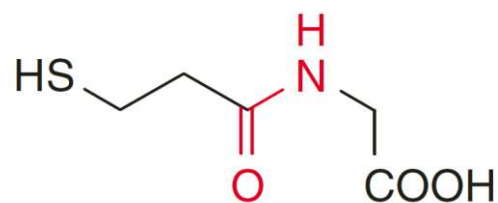
25.14

$IC_{50} = 17 \mu M$



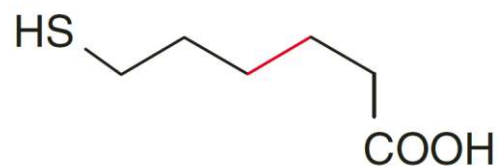
25.15

$IC_{50} = 4300 \mu M$



25.16

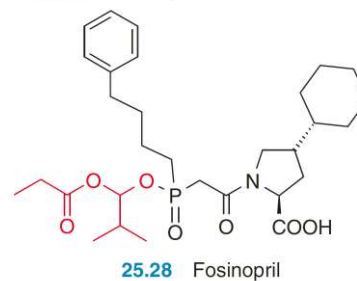
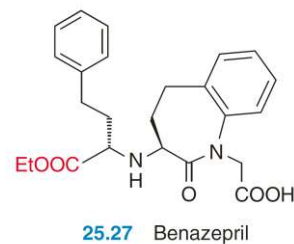
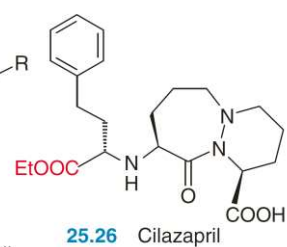
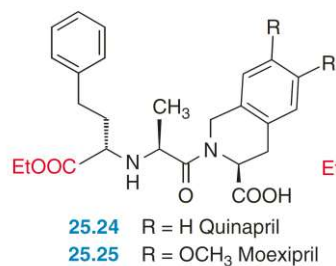
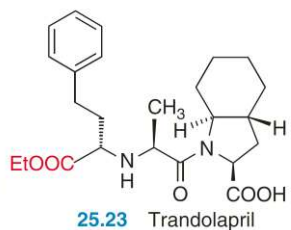
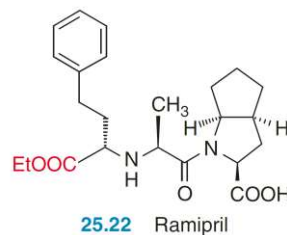
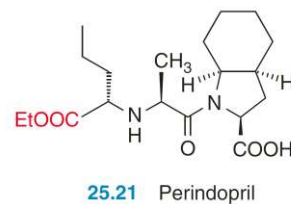
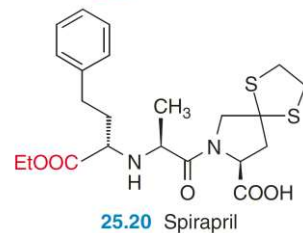
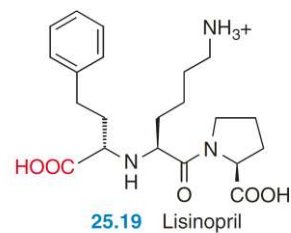
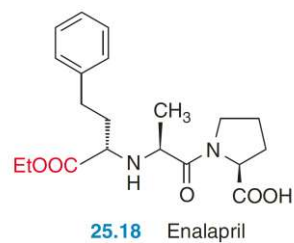
$IC_{50} = 2.8 \mu M$



25.17

$IC_{50} = 1100 \mu M$

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



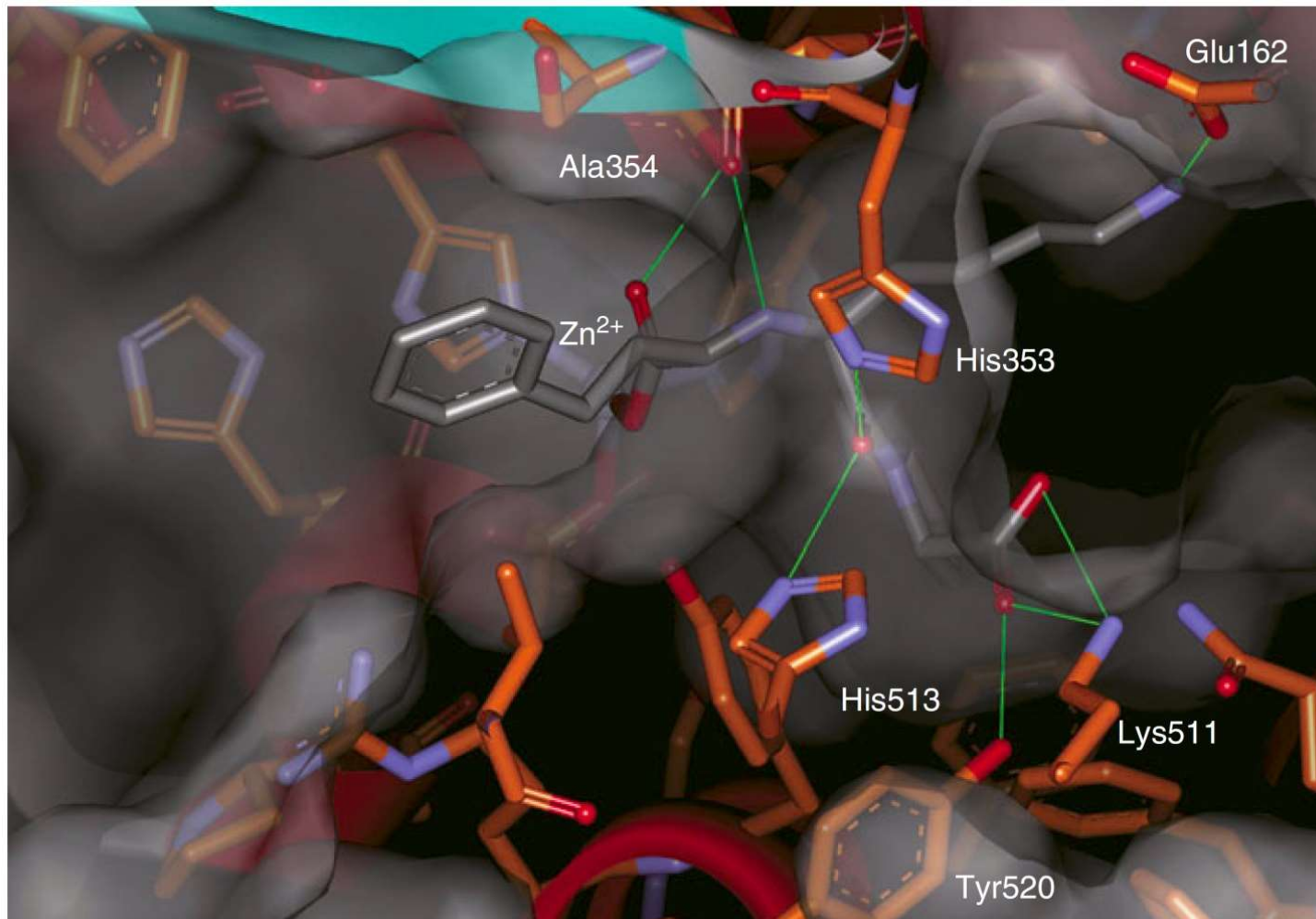
Outros inibidores da ACE desenvolvidos posteriormente.

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
- A degradação da bradicinina ocorre preferencialmente num dos domínios e a inibição deste processo pode assim, potencialmente, ser desacoplada da inibição da síntese da Angiotensina II
- A busca de inibidores específicos para domínio é um dos aspectos mais importantes no desenvolvimento de novos inibidores da ACE

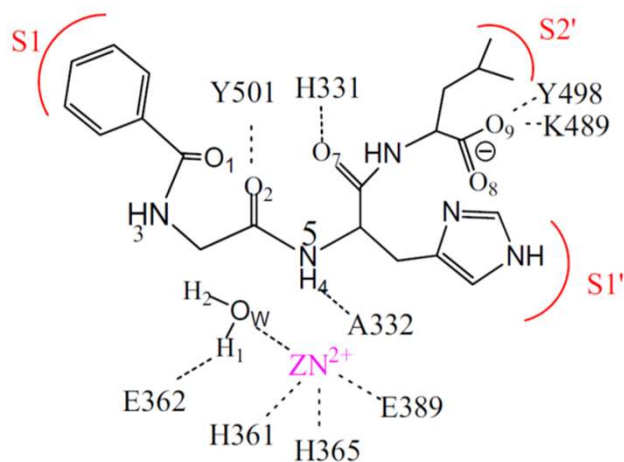
Em 2003 o grupo de Edward Sturrock determinou a estrutura cristalográfica da t-ACE em complexo com o lisinopril. Posteriormente foi resolvida a estrutura da s-ACE, mostrando a presença de dois domínios com actividade catalítica (domínio N-terminal e domínio C-terminal) e sequências diferentes (~50% de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



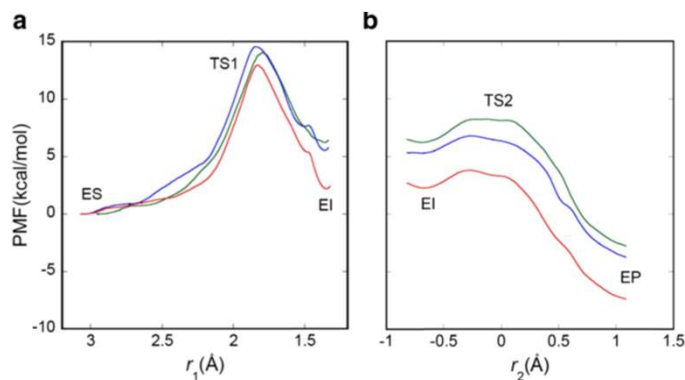
t-ACE em complexo com o **lisinopril**

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

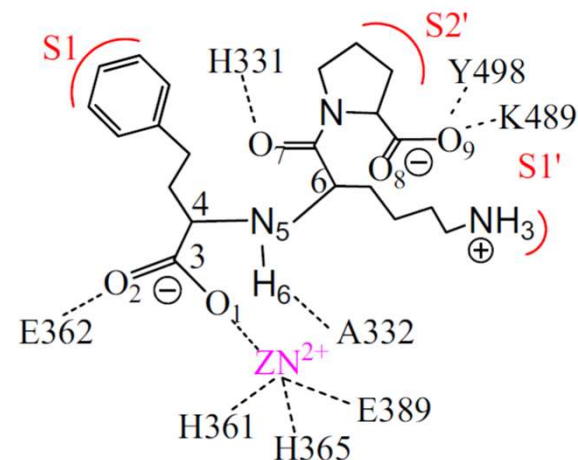
Mecanismo catalítico da ACE (hipotético)



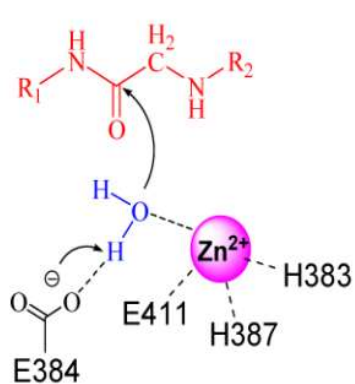
Hip-His-Leu



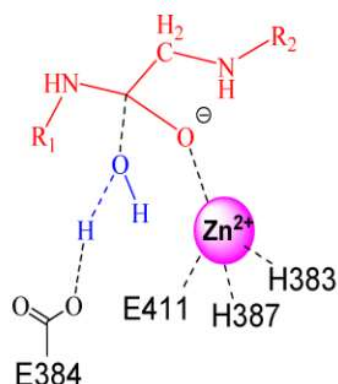
Perfil de energia da reacção



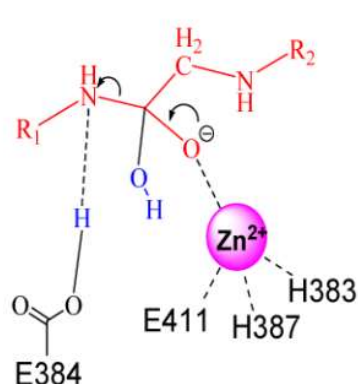
Lisinopril



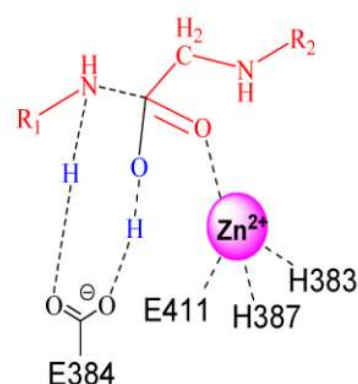
ES



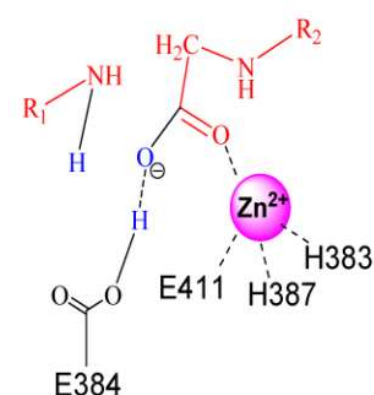
TS1



EI

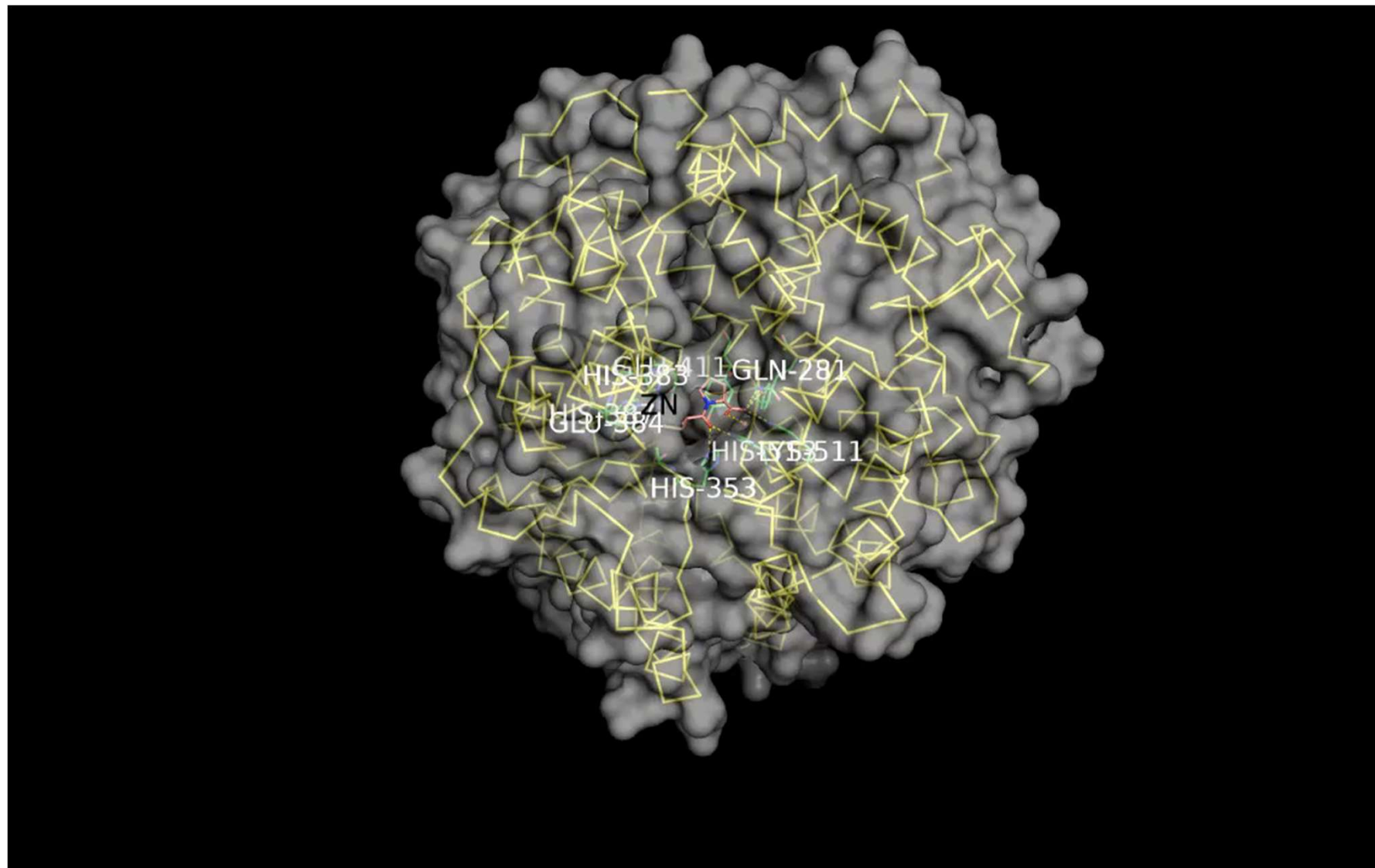


TS2

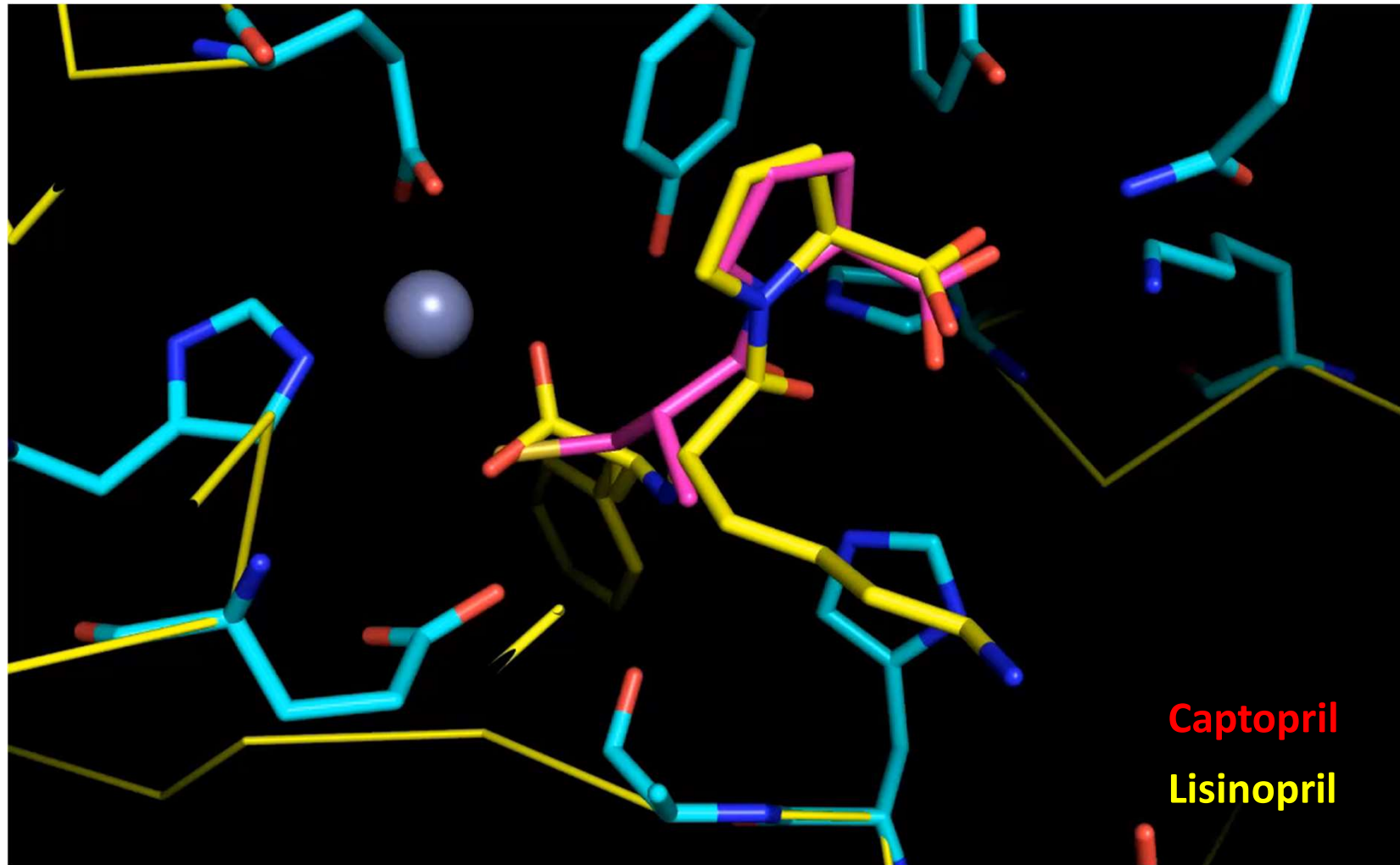


EP

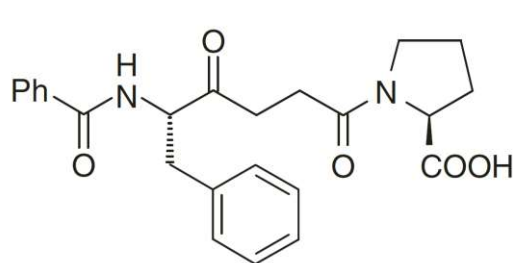
t-ACE humana em complexo com o captopril



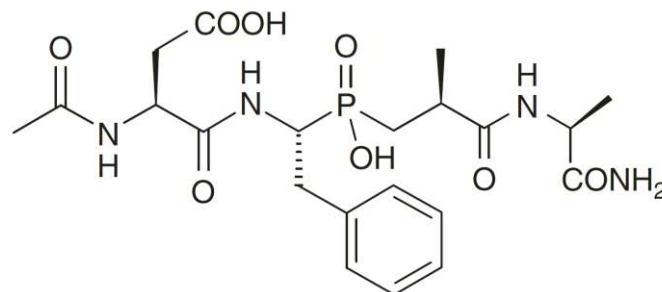
Comparação do captopril e lisinopril no centro active da t-ACE



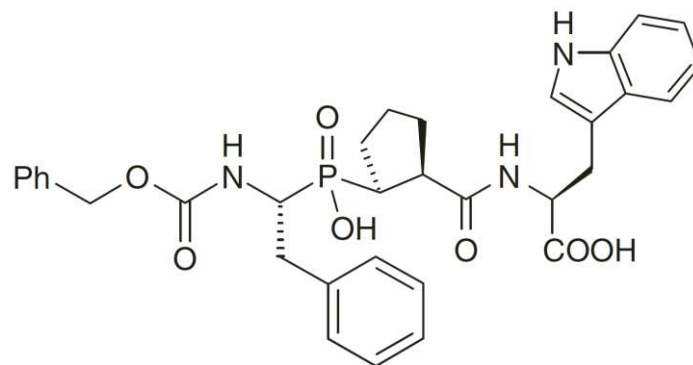
Inibidores específicos para os domínios N e C da ACE



25.29 Keto-ACE



25.30 RXP407



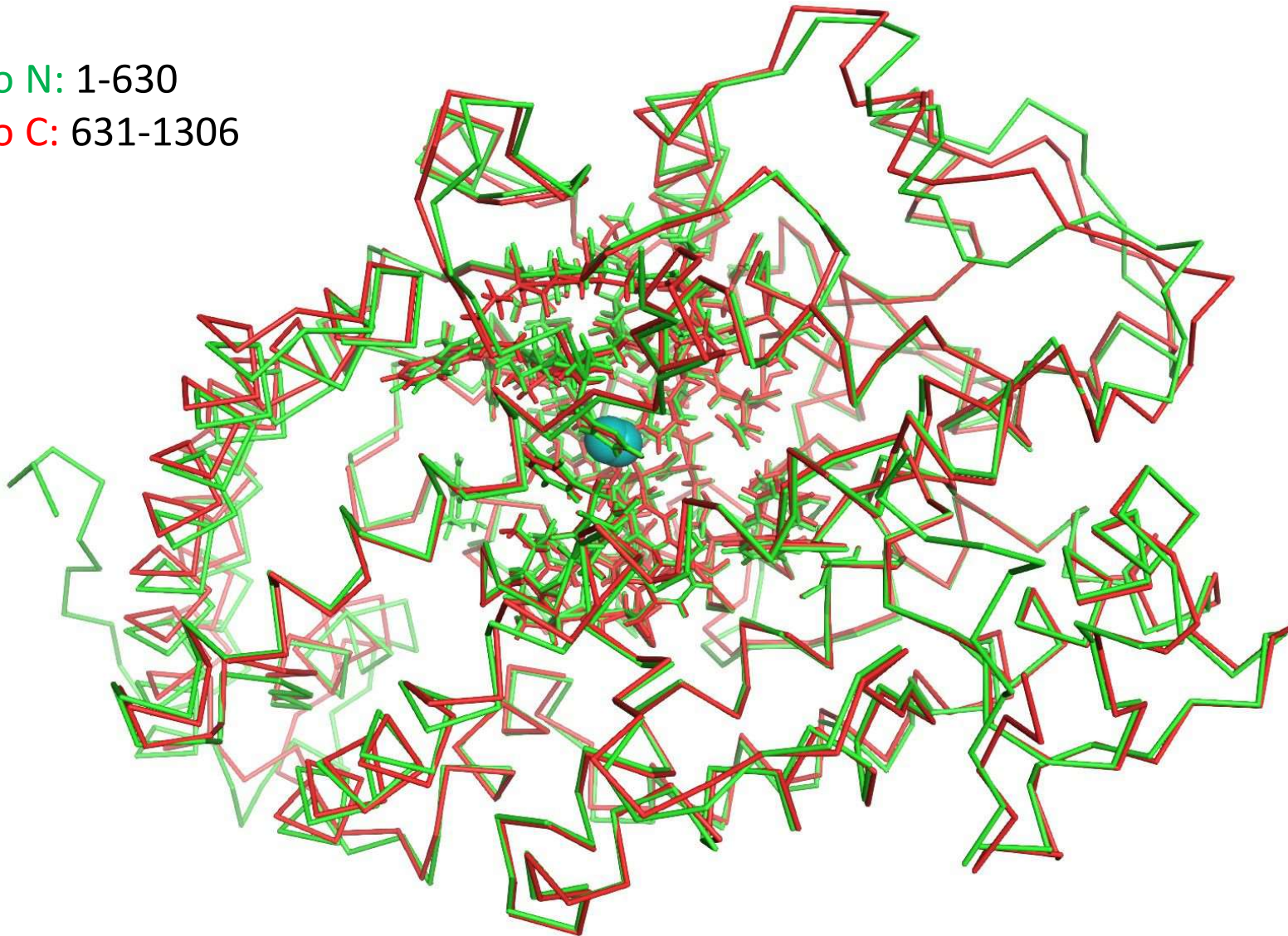
25.31 RXPA380

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

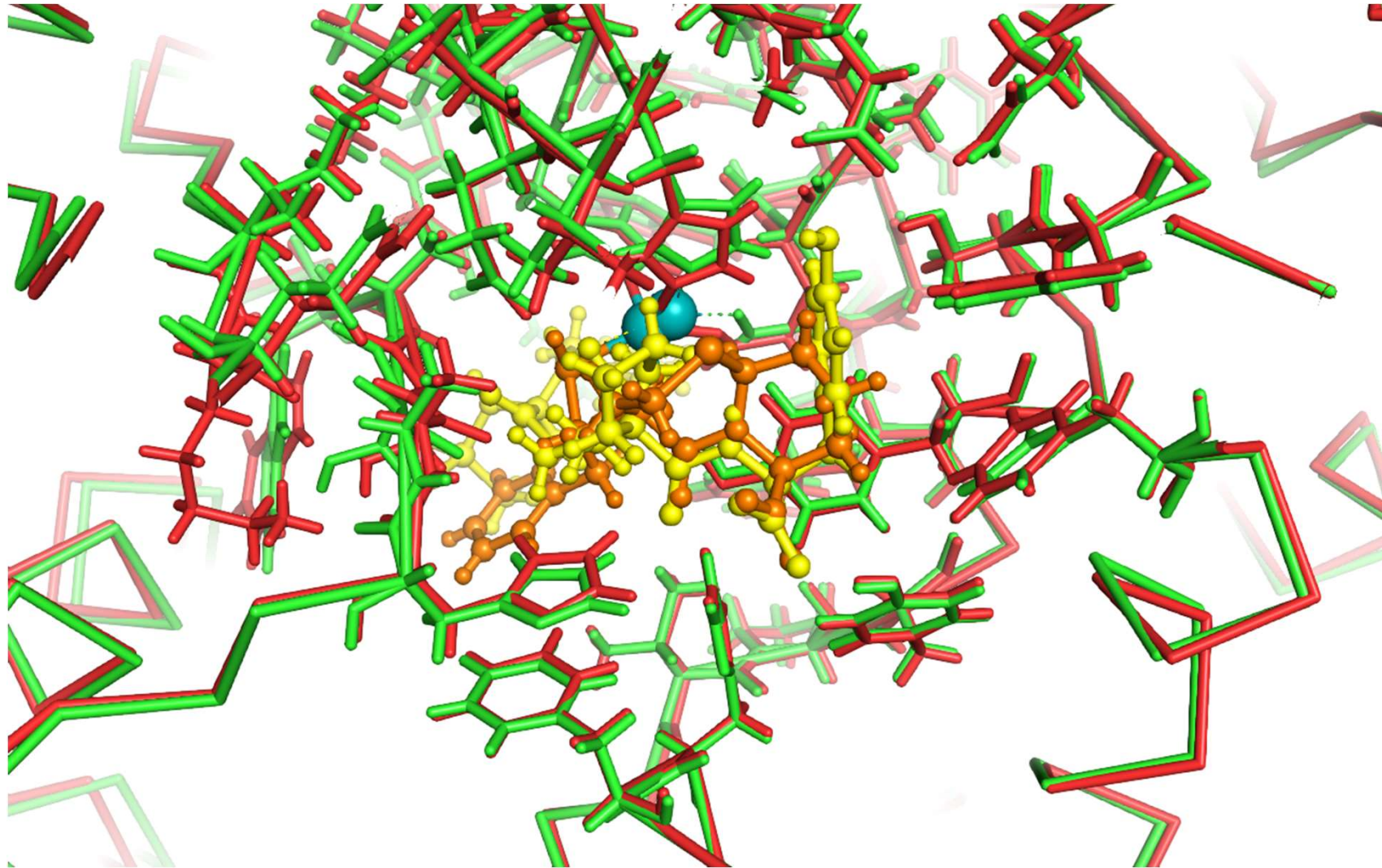
Domínio C: 631-1306



Percentage de identidade entre os domínios: **53%**

PDBs: **6H5W, 6F9V**

s-ACE humana



Sampatrilat

Omapatrilat

Domínio N: 1-630

Domínio C: 631-1306

Geração e pesquisa de uma biblioteca de tripéptidos inibidores da ACE

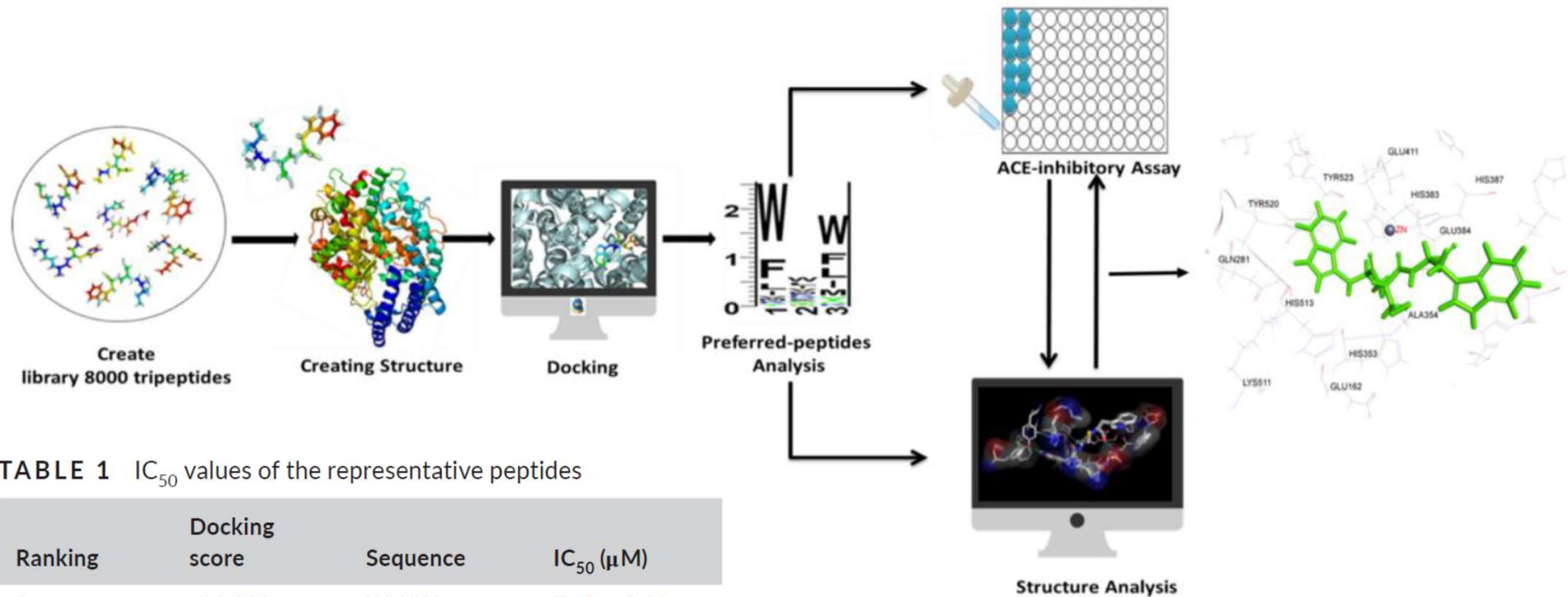


TABLE 1 IC_{50} values of the representative peptides

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 \pm SD.

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

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