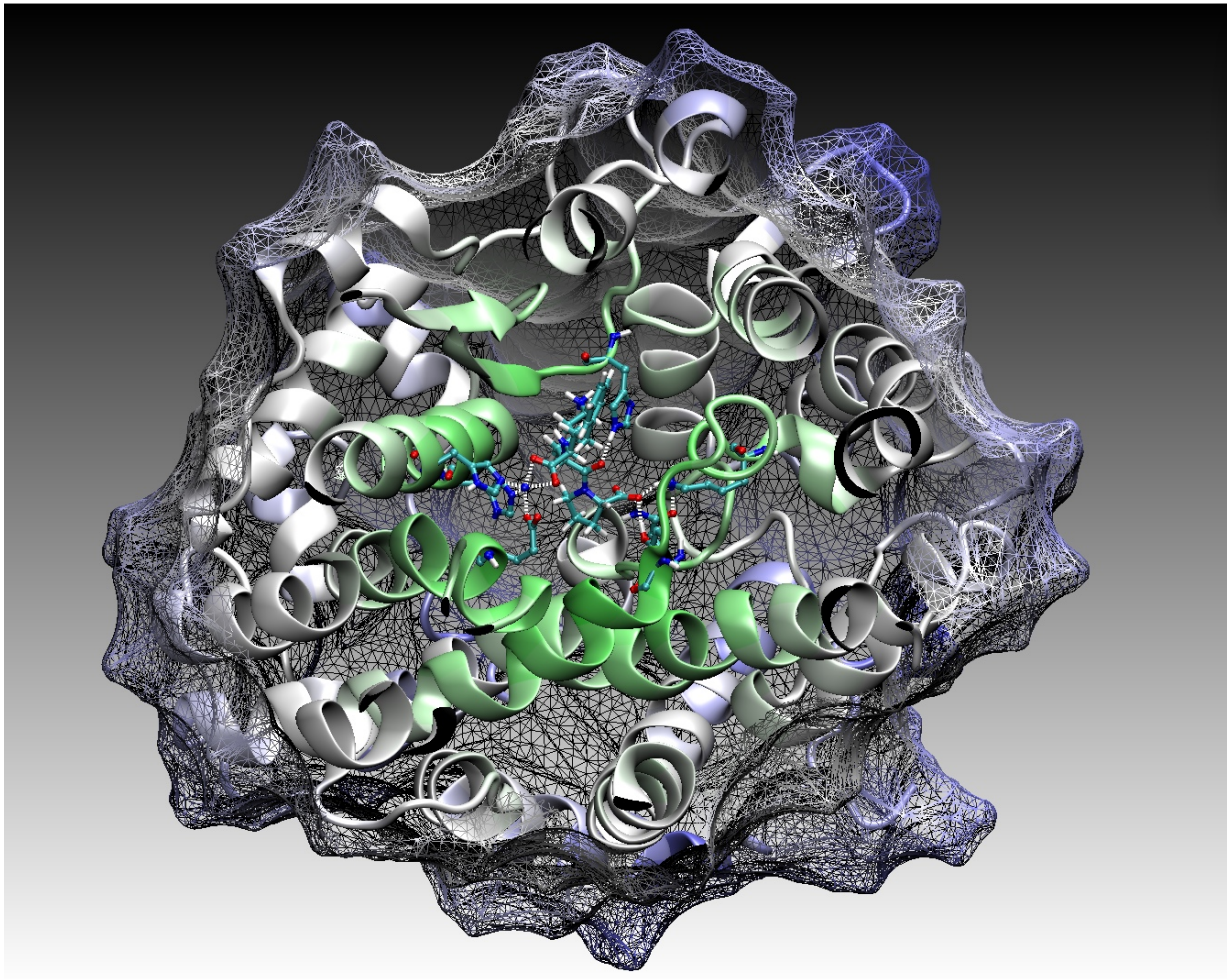
Structure-Based Design



Structure-Based Design (SBD)

State-of-the-art approach in the Computer-Aided Drug Design applied when **3D structure** of the **target** protein **is known** (thanks to a rapid advance of protein purification and crystallography methods, robotics and automation).

Applicability domain

- projects in which 3D structure of the receptor (with a ligand) is known

Examples of drugs designed by SBD:

- carbonic anhydrase-II inhibitors (434 crystal structures in PDB, since 1990!!!)
- antivirotics: Indinavir - HIV protease inhibitor, Zanamivir – neuraminidase inhibitor
- kinase inhibitors (challenging due to target flexibility)
- domain selective ACE inhibitors → case study

Ligand-based design

3D structure of the target protein is **unknown**

screening study

↓

hit(s)

↓

conformational search

superposition

pharmacophore

ligand optimization / database searching

scoring: ***rmsd*, QSAR, CoMFA**, etc.

↓

assay

↓

lead compound / drug candidate

Structure-based design

3D structure of the target protein is **known**

target purification & crystallization

↓

protein(-ligand) 3D structure

↓

molecular docking

pharmacophore

ligand optimization / database searching

scoring: **generic function, QM-MM** etc.

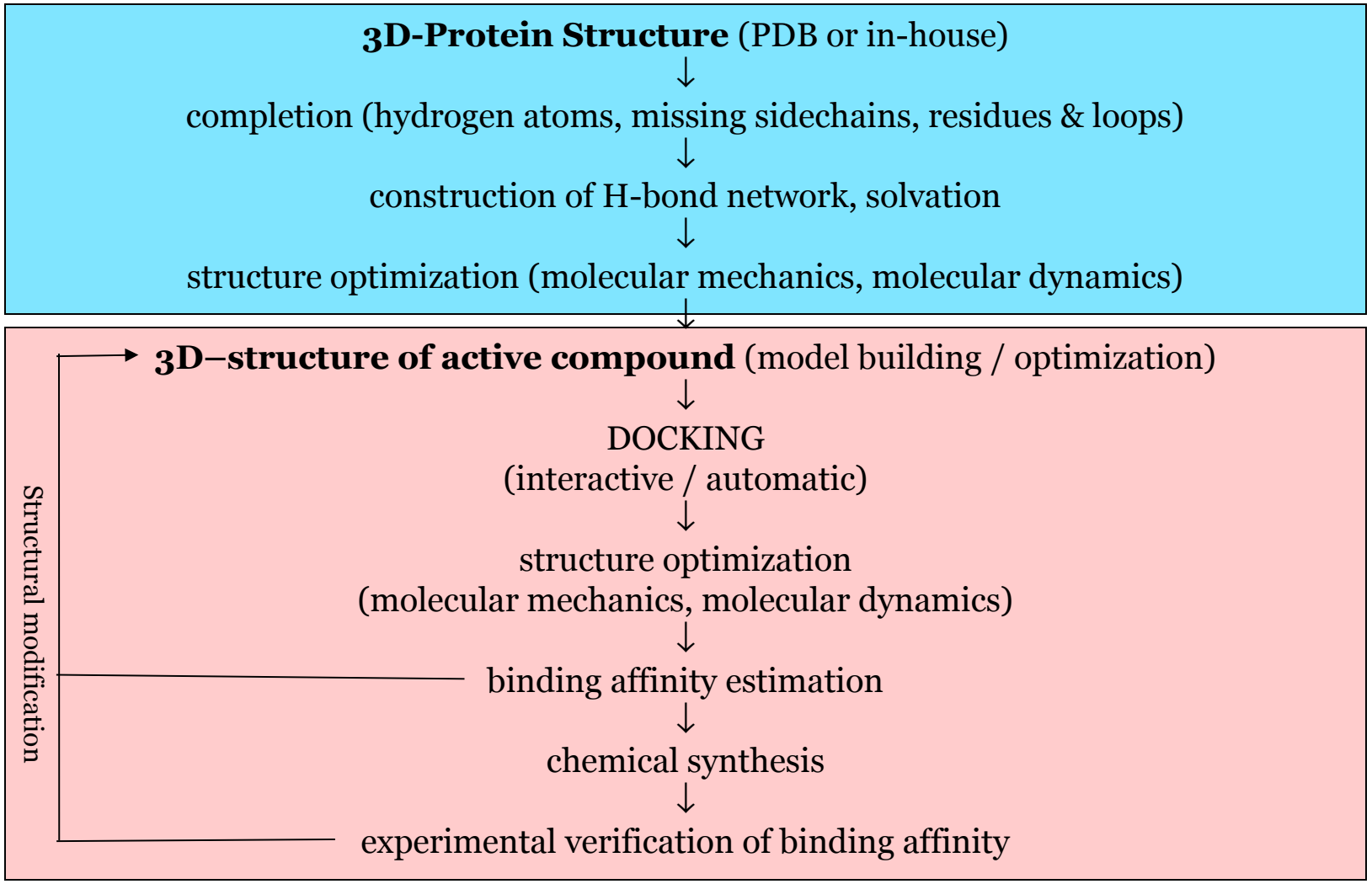
↓

assay

↓

lead compound / drug candidate

Structure-Based Design: Workflow



SBD: Typical procedure

1. Detailed analysis of known ligand–protein complexes

- binding site (where is?)
- binding mode (H-bonds, metals, hydrophobic interactions)
- pharmacophore: which are the most important residues
- what are competitors doing (which compound classes are already patented)?

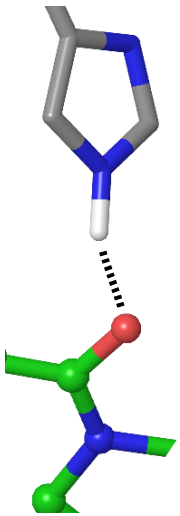
2. Rational design of a novel active molecule (creative act or database search)

- feasible synthesis
- metabolic stability, bioavailability, solubility, toxicity (ADMET → next week)
- protonation state at the site of action

3. Building 3D-structure model

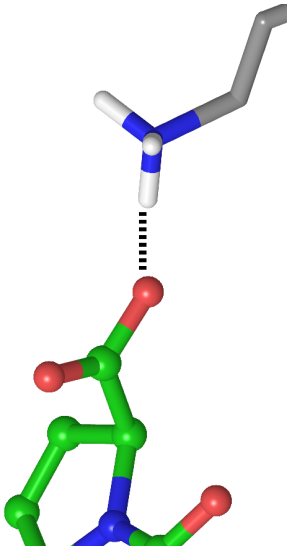
- optimizing 3D-structure (Molecular Mechanics)
- conformational search in water (global minimum + low energy conformers)
- calculation of partial atomic charges (quantum chemistry)

SBD : Hydrogen bonds & Salt bridges



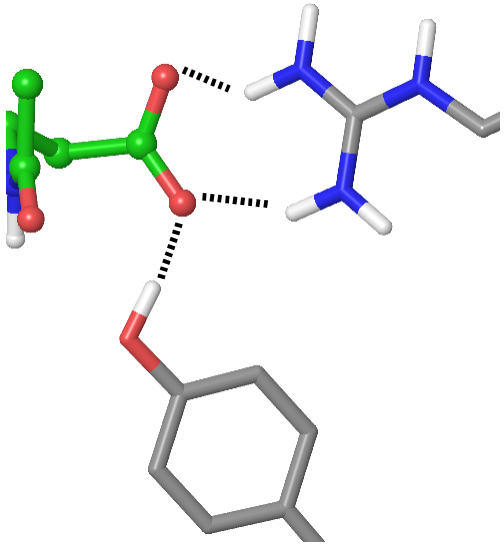
H-bond

neutral



Salt bridge

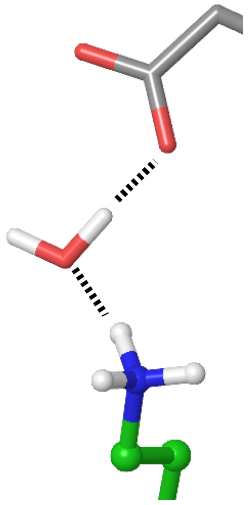
-COO^{-}····$^{+}$Lys-



Salt bridge

-COO^{-}····$^{+}$Arg-

(plus an H-bond)

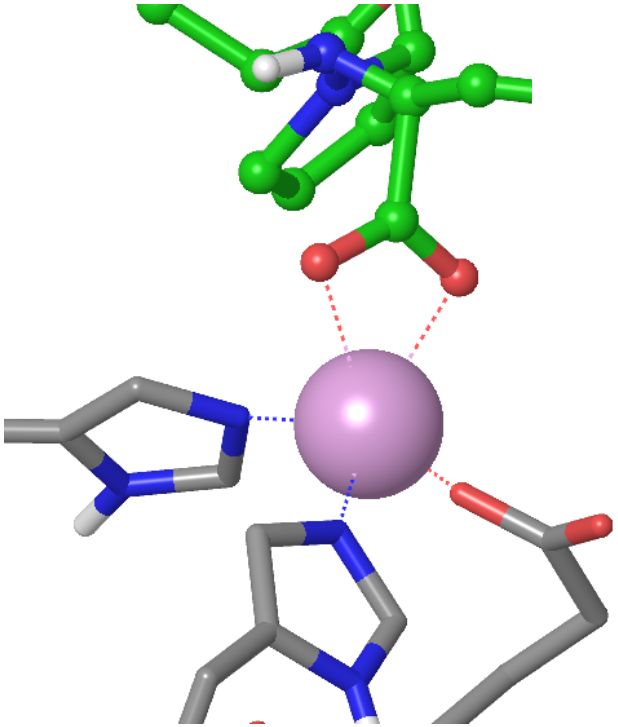


Salt bridge

-NH$_3^{+}$····H$_2$O····$^{-}$Asp-

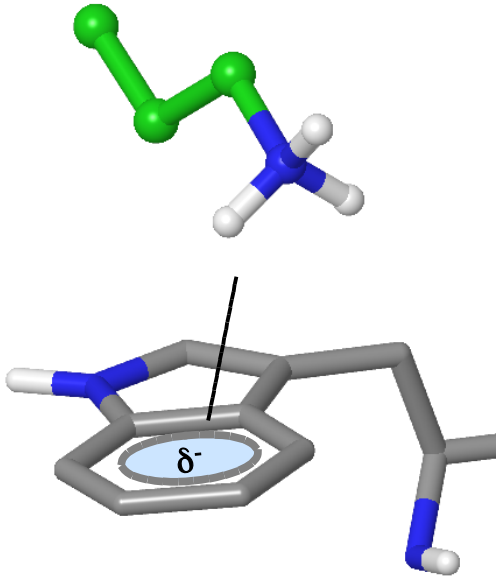
water mediated

SBD : special interactions



metal····ligand interaction

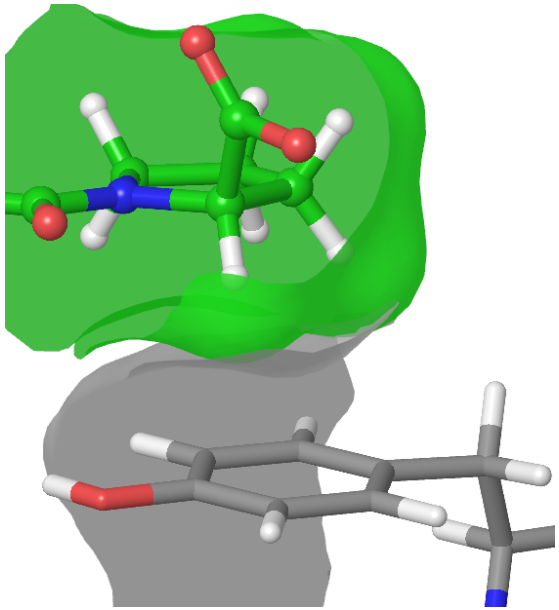
(2xHis, 1xGlu) Zn^{2+}····$^{-}$OOC-R



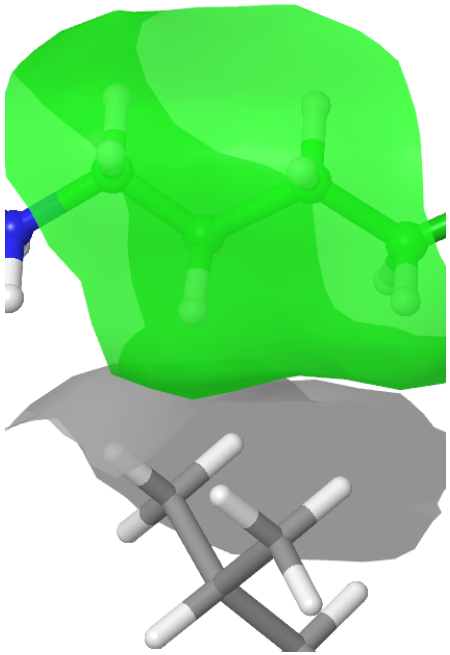
charge···˜ π-system interaction

R-NH_3^+····Trp-

SBD : hydrophobic interactions



π-system····aliphatic ring



aliphatic chain····aliphatic chain

SBD: Typical procedure (*continued*)

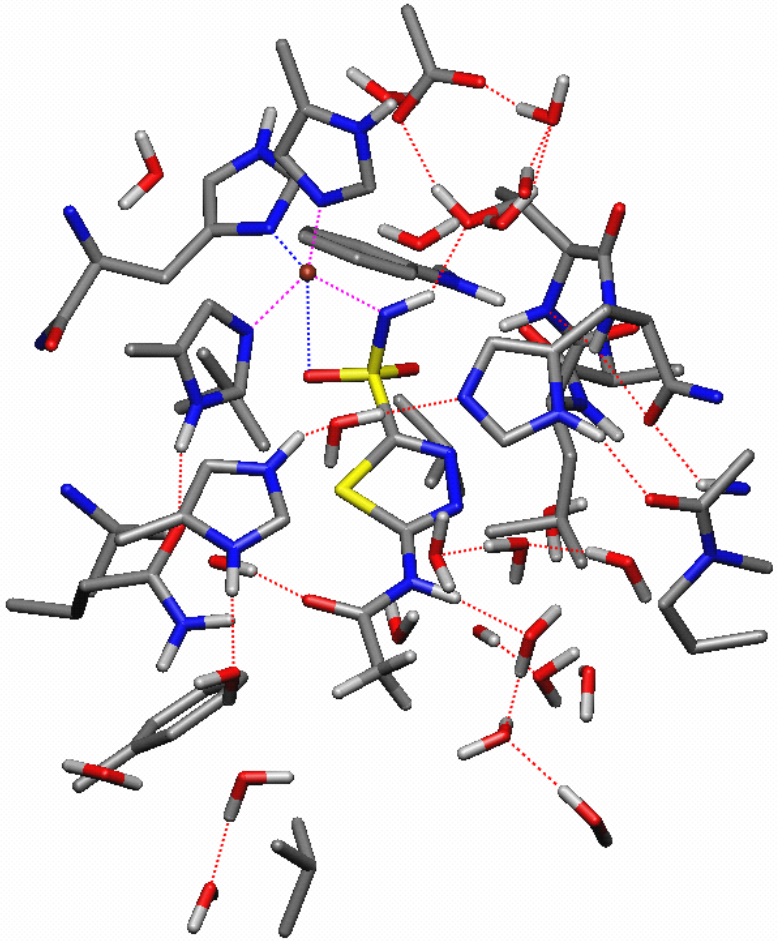
4. Molecular Docking – computational technique for the exploration of the possible binding modes of a molecule to a given receptor, enzyme or other binding site:

- interactive (manual) – requires experienced chemist, hardly reproducible
- automatic (looking for a needle in a haystack…)
- rigid (fast, not too reliable) or flexible (slow, problematic)
- advanced docking methods:
  - pharmacophore pre-alignment (Praktikum)
  - placing fragments into preferred position and linking them together

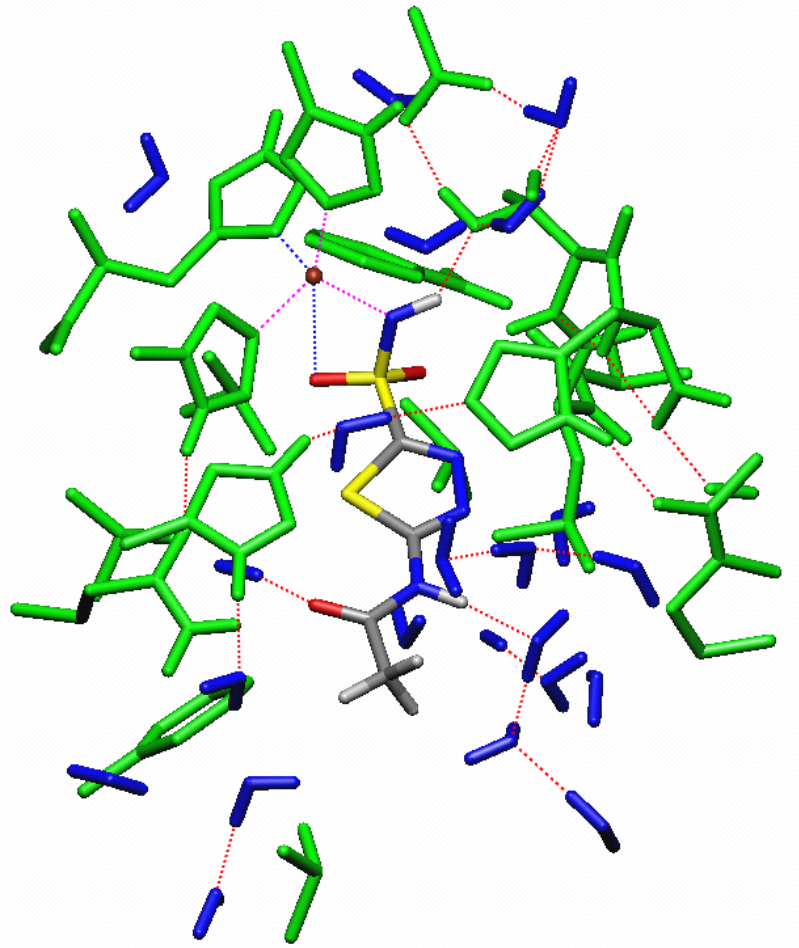
5. Scoring

- direct scoring calculation of the binding free energy using force-field terms (or in combination with quantum mechanics → QM-MM methods)
- specialized scoring function containing empirical terms (e.g. polar surface area)
- QSAR models

SBD: Interactive molecular docking

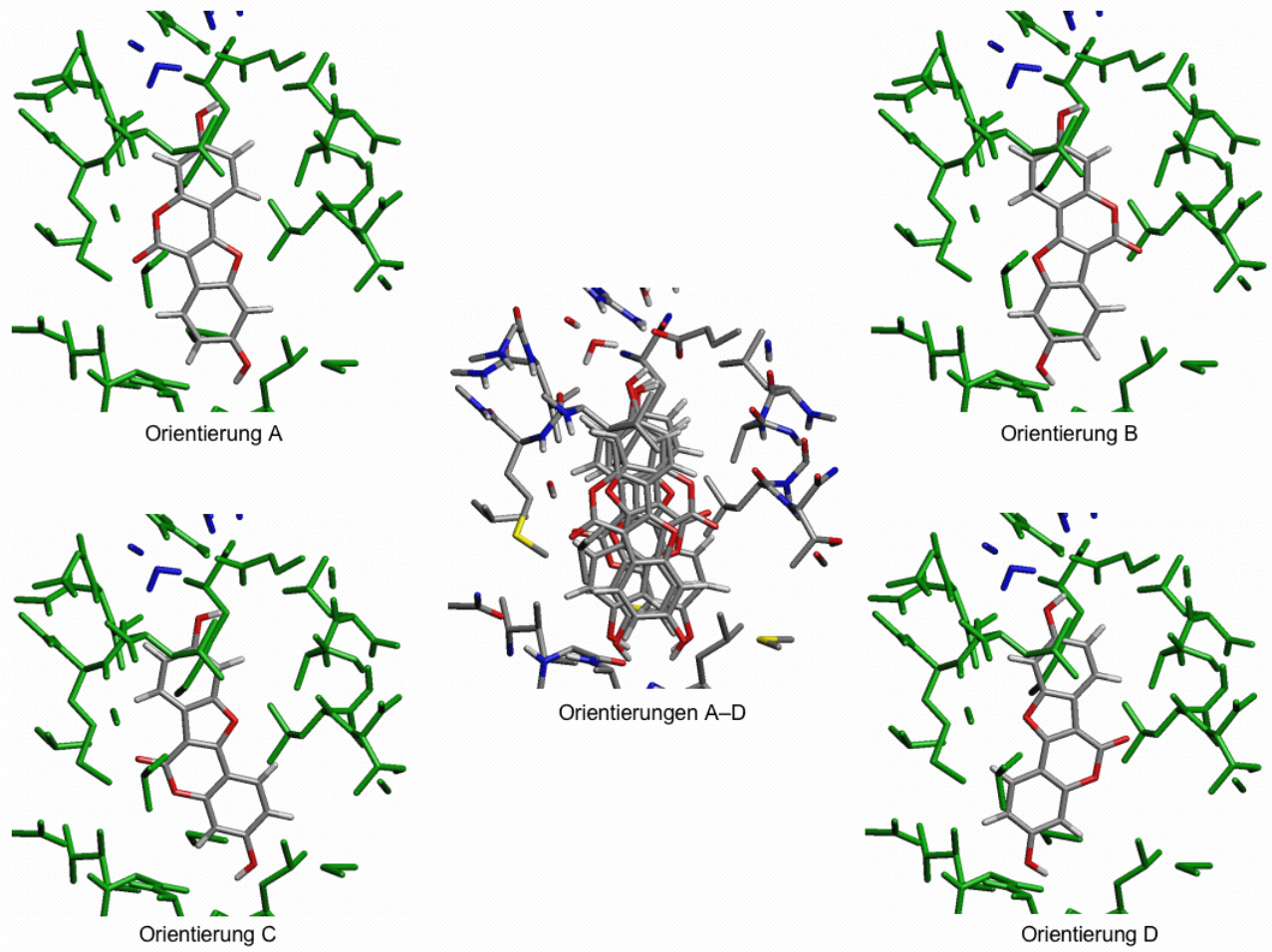


Coloring by atom types



Coloring: ligand → by atom types, protein → green, water → blue

SBD: Automatic molecular docking



Identification of all (?) binding modes

Optimization of the ligand–protein-interactions

$$E_{total} = \sum_{bonds} K_r (r - r_{eq})^2 + \sum_{angles} K_\theta (\theta - \theta_{eq})^2 + \sum_{torsions} \frac{V_n}{2} [1 + \cos(n\phi - \gamma)] +$$

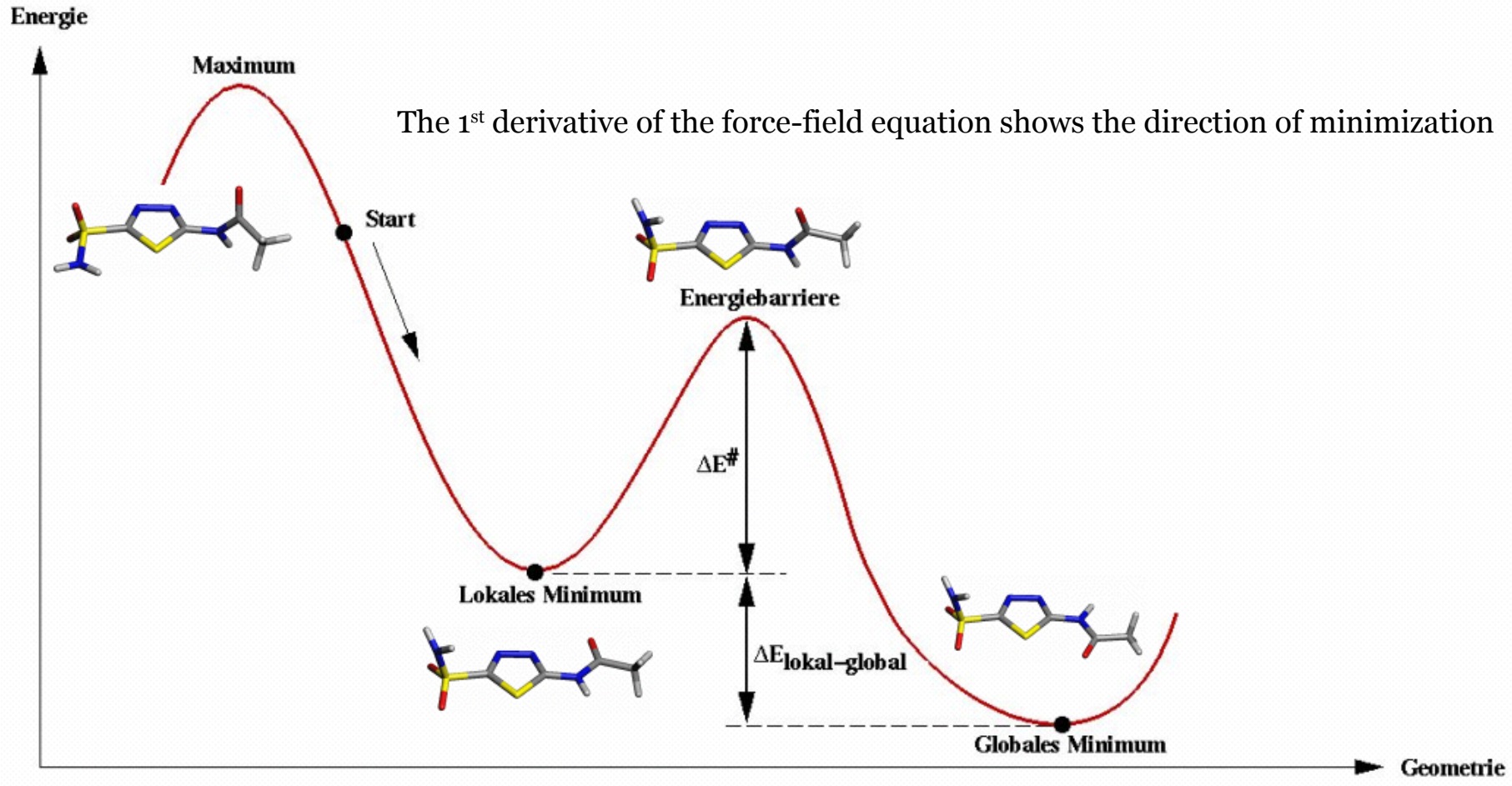
$$\sum_{nb\ pairs} \frac{q_i \cdot q_j}{4\pi\varepsilon_0 D(r) r_{ij}} + \sum_{nb\ pairs} \left(\frac{A}{r_{ij}^{12}} - \frac{B}{r_{ij}^{6}} \right) +$$

$$\sum_{H\ bonds} \left(\frac{C}{r_{ij}^{12}} - \frac{D}{r_{ij}^{10}} \right) \cdot \cos^2(\theta_{Don-H\cdots Acc}) \cdot \cos^n(\omega_{H\cdots Acc-LP}) +$$

$$\sum_{metal\ pairs} \frac{q_i^{CT} \cdot q_j^{CT}}{4\pi\varepsilon_0 D(r) r_{ij}} + \sum_{metal\ pairs} \left(\frac{E}{r_{ij}^{12}} - \frac{F}{r_{ij}^{10}} \right) +$$

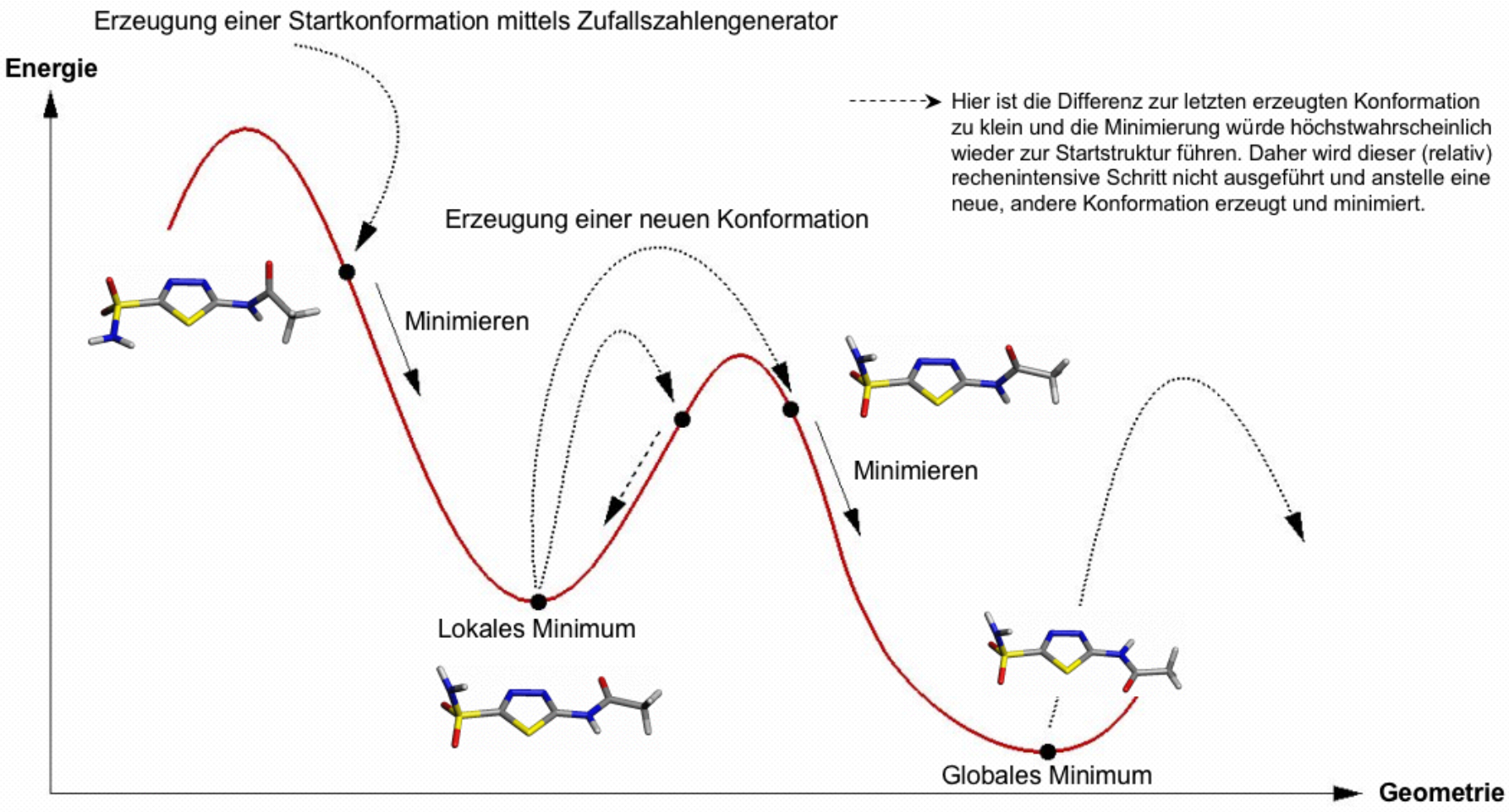
$$(E_{MC} + E_{LFS}) \cdot \prod_{angles} \cos^2(\Psi_{Lig-Met-Lig'} - \Psi_{eq}) \cdot \frac{1}{n} \sum_{ligands} \cos^n(\omega_{Met\cdots Lig-LP})$$

Structure optimization: Molecular Mechanics



Molecular Mechanics optimizations end up always in the nearest local minimum

Structure optimization: Conformational search



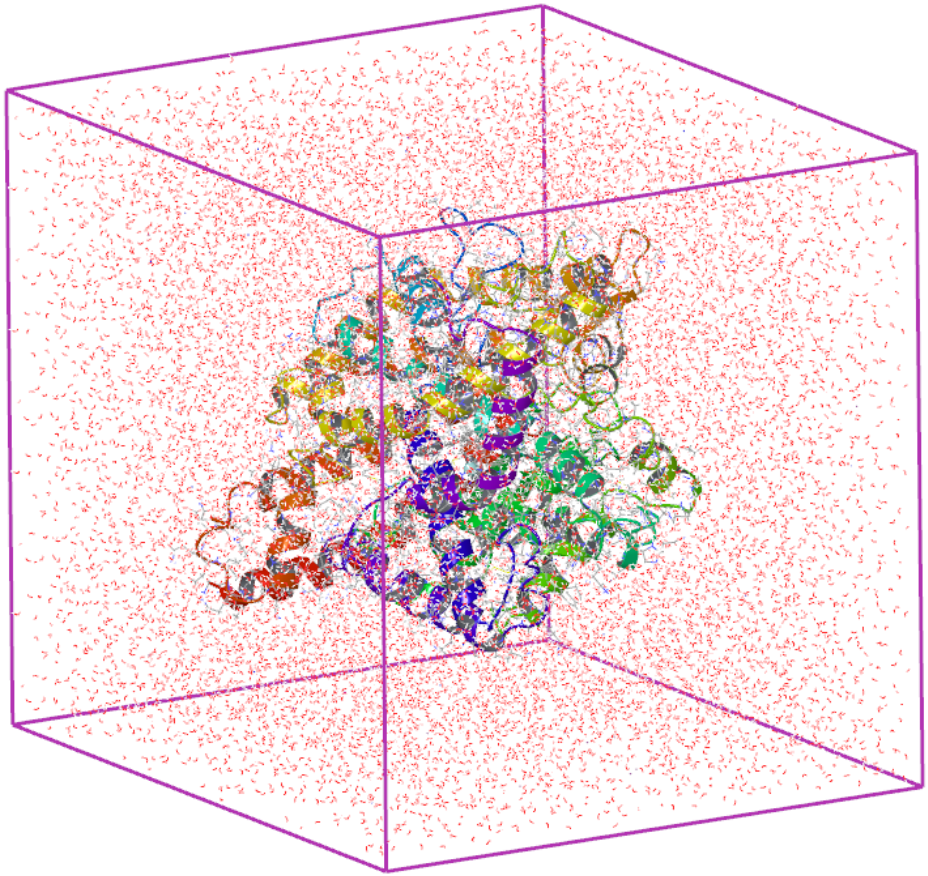
Structure optimization: Molecular Dynamics

Advantages:

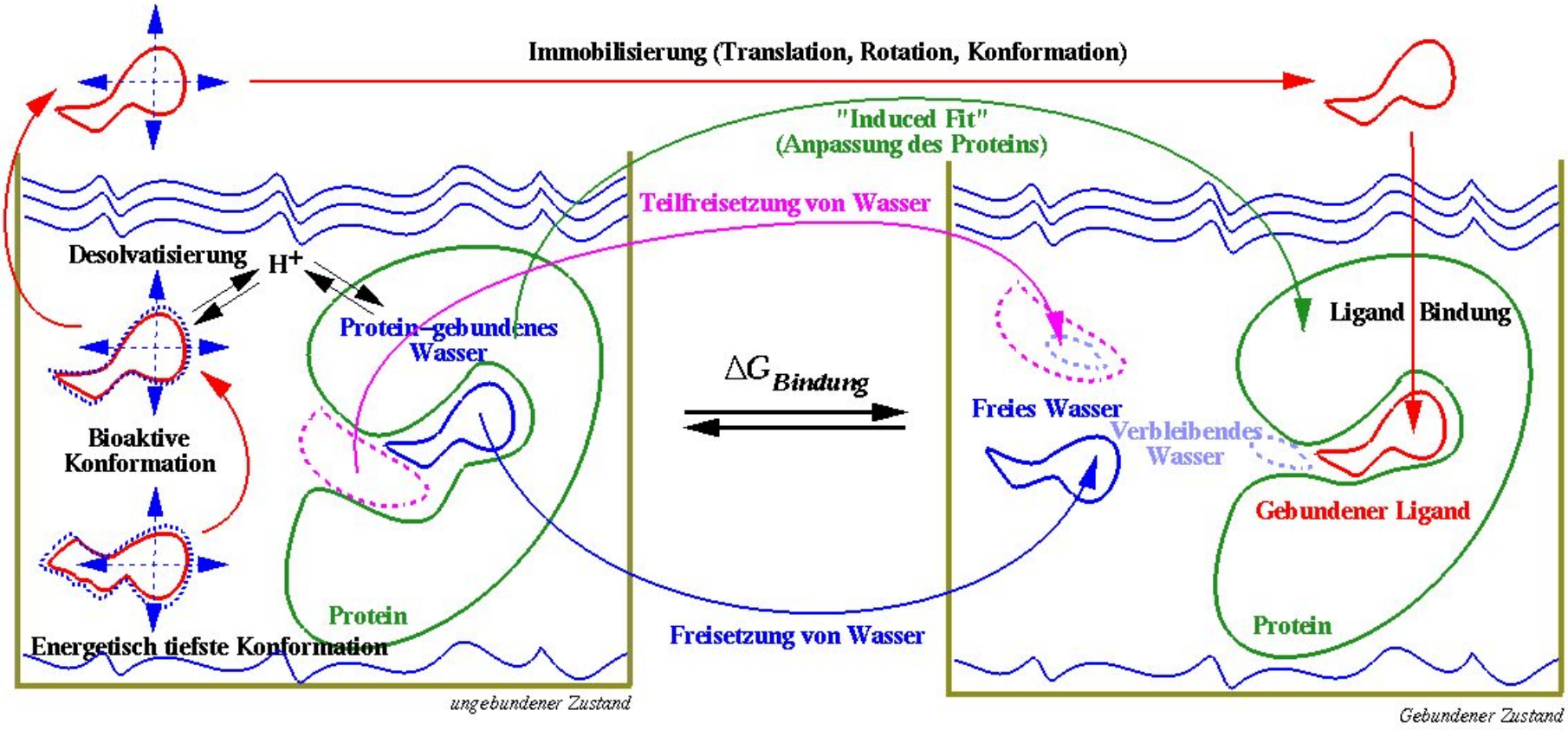
- strain relief
- simulation of induced-fit possible
- MD trajectory → information about dynamic stability of intermolecular interactions
- time-averaged (representative) structure
- explicit solvent

Drawbacks:

- risk of introducing artifacts
- time consuming



Structure optimization: Scoring



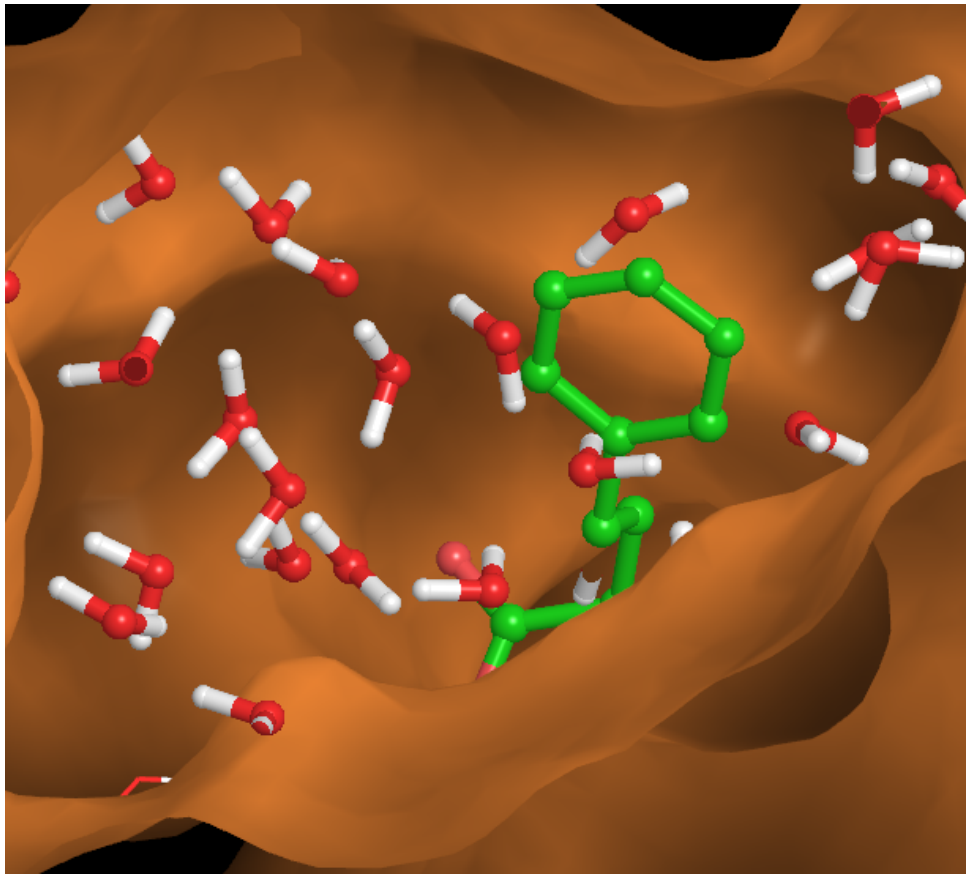
$$E_{Binding} = E_{Ligand-Protein} + E_{Ligand-Solvent} - E_{Internal\ Strain} - E_{Ligand\text{-}Desolvation} - T\Delta S$$

Structure optimization: $E_{Ligand-Protein}$

- hydrophobic interactions
- displacing protein-bound water (entropic effect)
- salt bridges
- interactions with metals
- double H-bonds
- carbonyl groups: >C=O
- nitrogen atoms in aromatic rings: –N=
- bridging water molecules
- hydroxyl groups: –OH (3 H-bonds in water)
- charged groups (exception: salt bridges, metals)

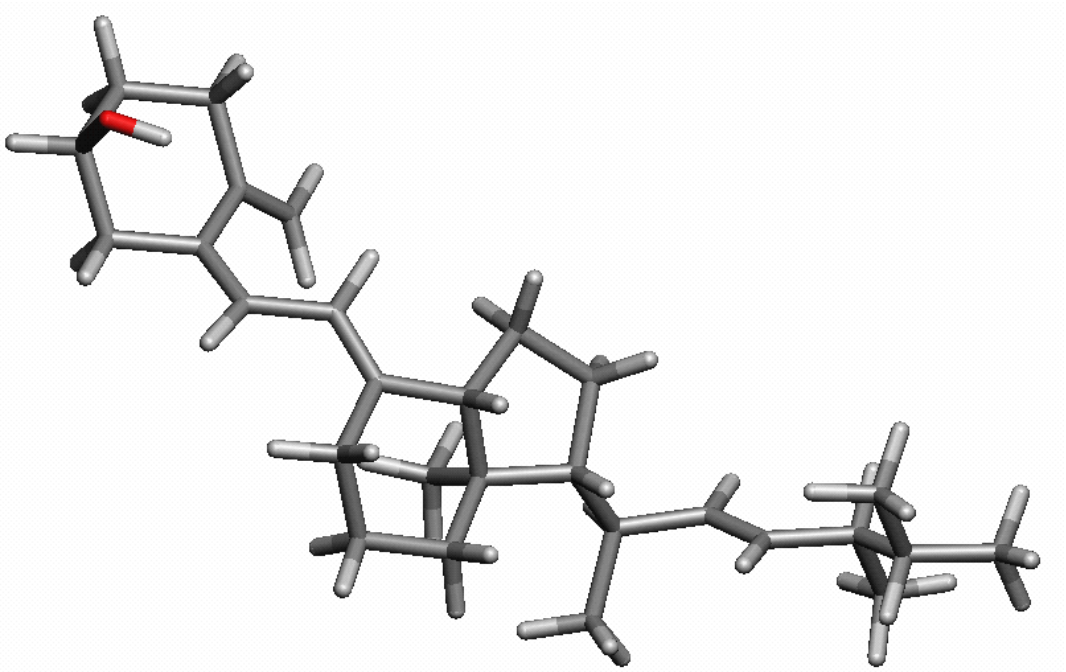
Important is the difference between the strength of interaction with water (in aqueous environment) and with protein (in the binding site) → ΔG

Structure optimization: $E_{Ligand–Solvens}$



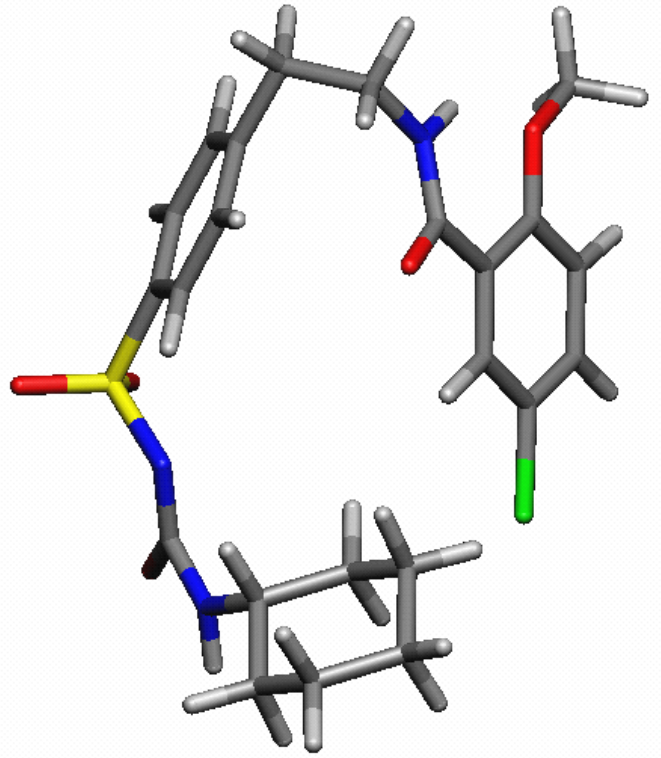
Solvent accessible binding site of the ACE (brown) with bound lisinopril (green) filled with water

Structure optimization: $E_{internal\ strain}$



Vitamin D_2 in the glucocorticoid-receptor: unstrained

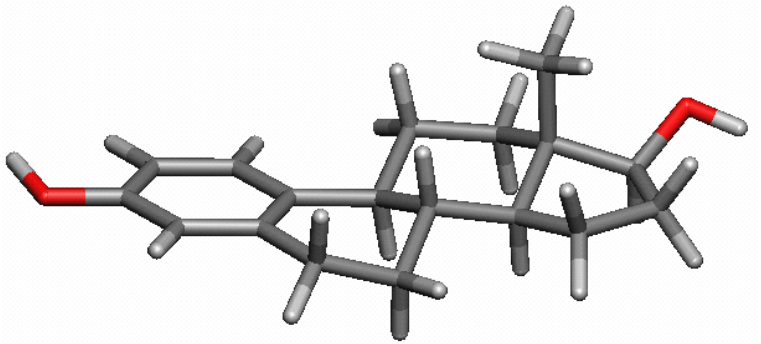
E_{int} = 1.2 kcal/mol



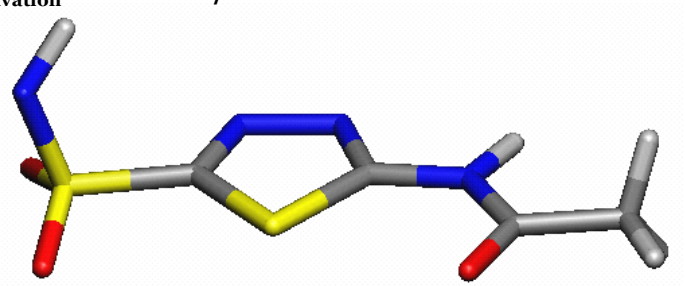
Glibenclamid in the Glucocorticoid-Receptor: strained

E_{int} = 10.8 kcal/mol

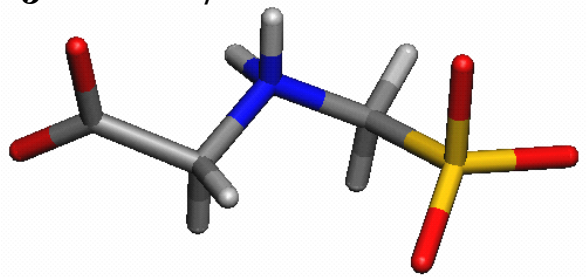
Structure optimization: $E_{Desolvation}$



neutral molecule (e.g. 17β-estradiol): **$E_{Desolvation}$ < 10 kcal/mol**

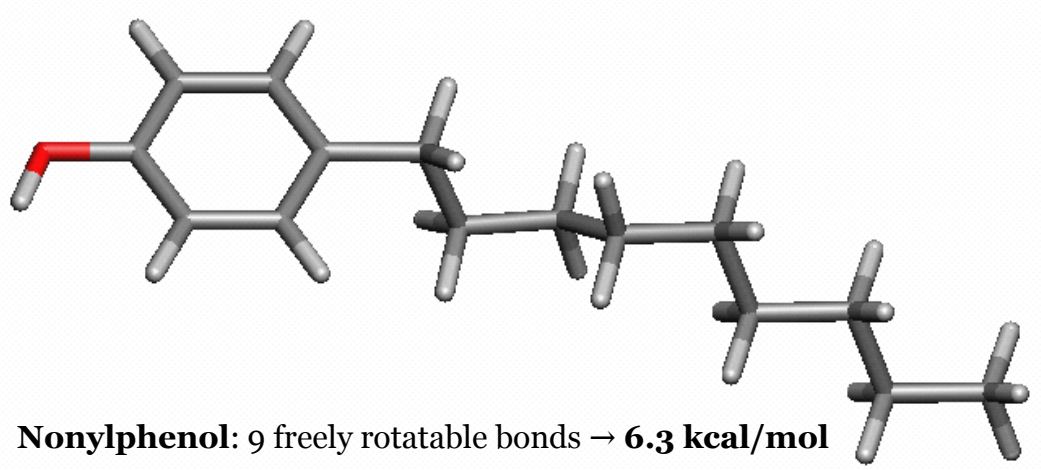


singly charged molecule (e.g. acetazolamid): **$E_{Desolvation}$ ≈ 50–60 kcal/mol**

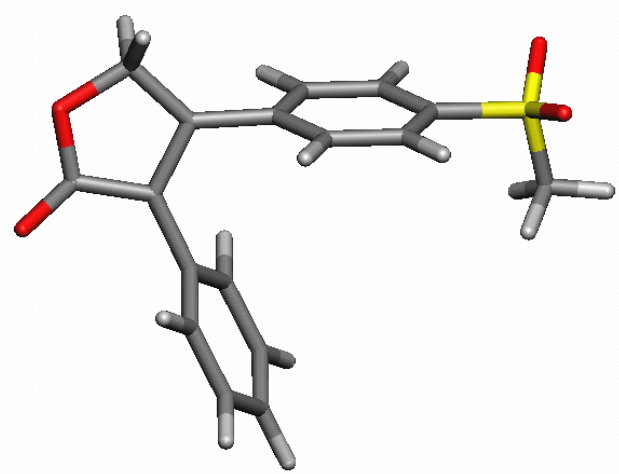


multiply charged molecule (e.g. glyphosat): **$E_{Desolvation}$ > 200 kcal/mol**

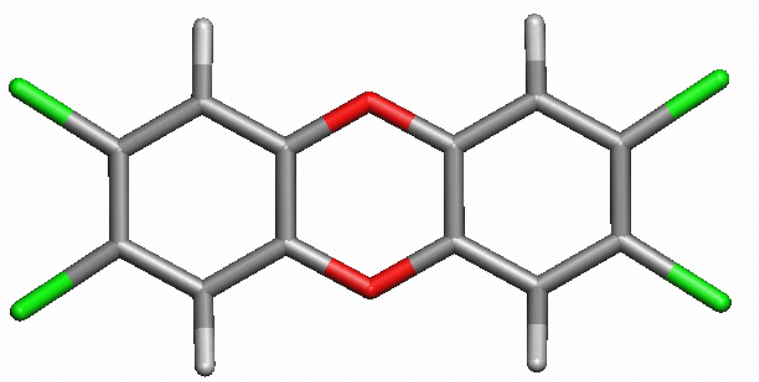
Structure optimization: TΔS



Nonylphenol: 9 freely rotatable bonds → **6.3 kcal/mol**

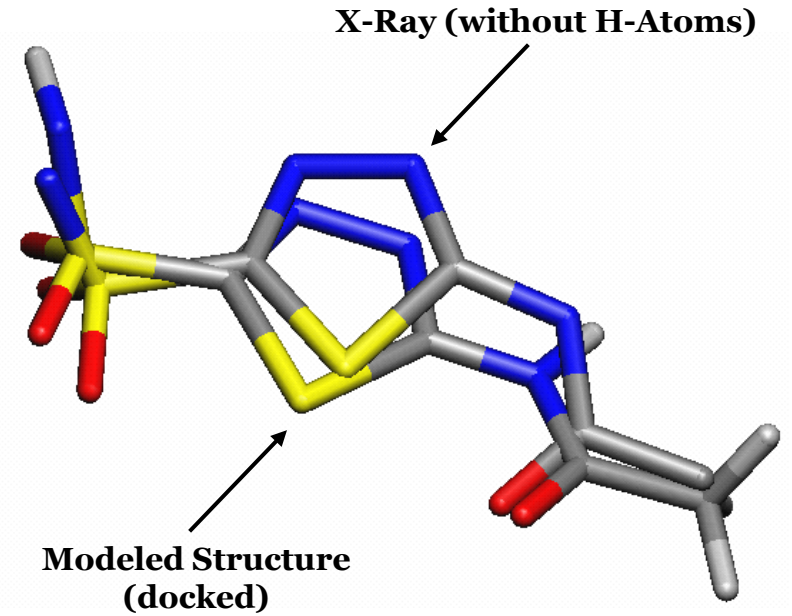


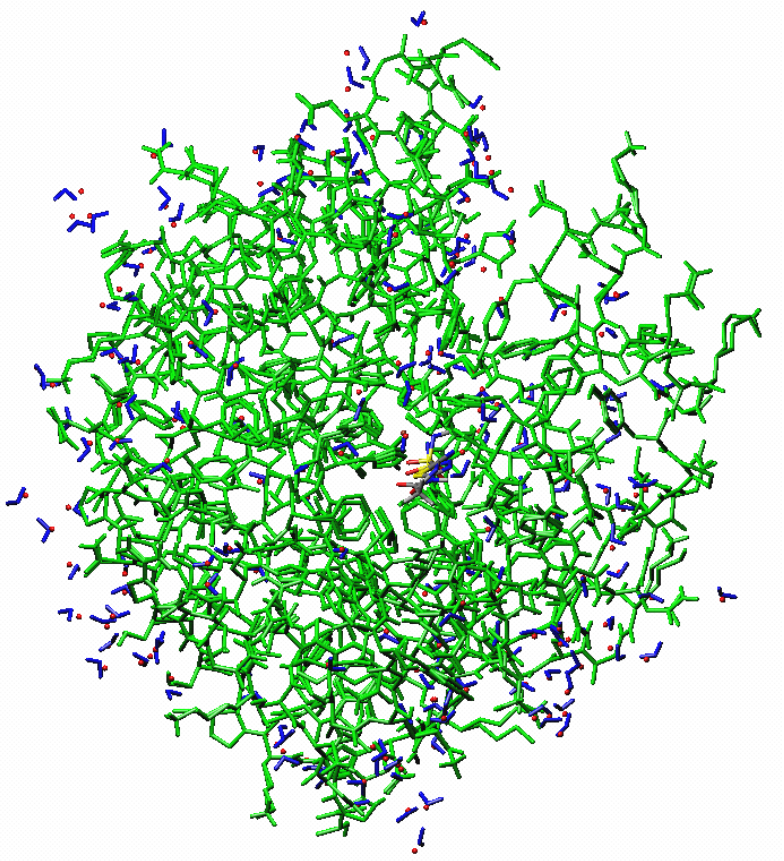
Rofecoxib: 3 freely rotatable bonds → **2.1 kcal/mol**



TCDD: no freely rotatable bonds → **0.0 kcal/mol**

Verification of the binding hypothesis





- **Comparison of the crystal structure (1993) with the predicted binding mode (1983 published)**
- **Calculated binding affinity (QSAR: 22.6 nM) – experimental value (14.1 nM)**

Structure Based Design - Conclusion

Advantages
- complete knowledge about the binding site → from (co)-crystal structure
- lower number of synthesized and tested compounds (rationally chosen structural changes)
- excellent complementarity with the binding site → good expected selectivity & safety
- virtual screening possible → novel ligands

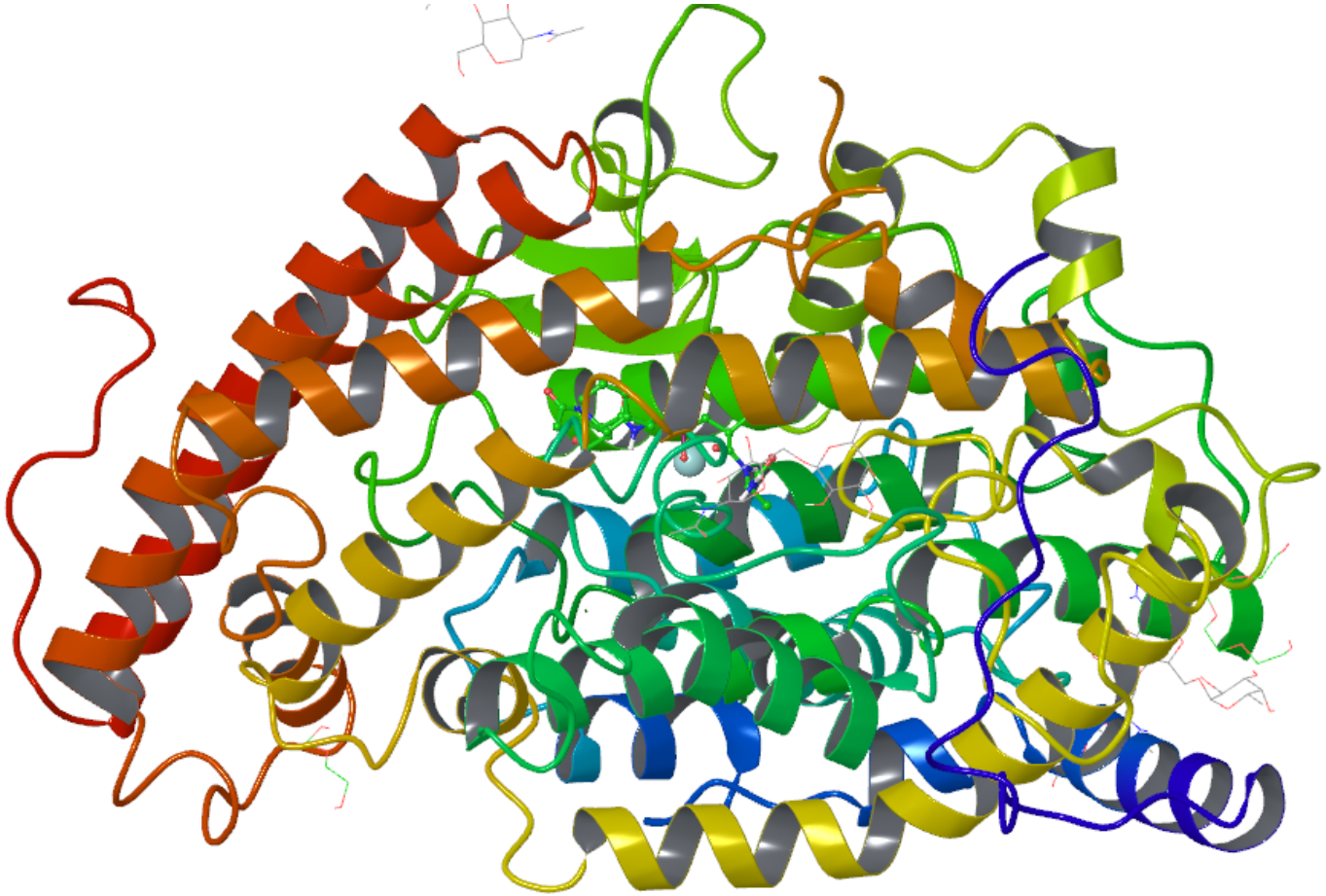
Disadvantages
- none

Prerequisites
- 3D structure of target
- good conformational search algorithm, force-field parameters, robust docking protocol, scoring function, consensus of multiple methods

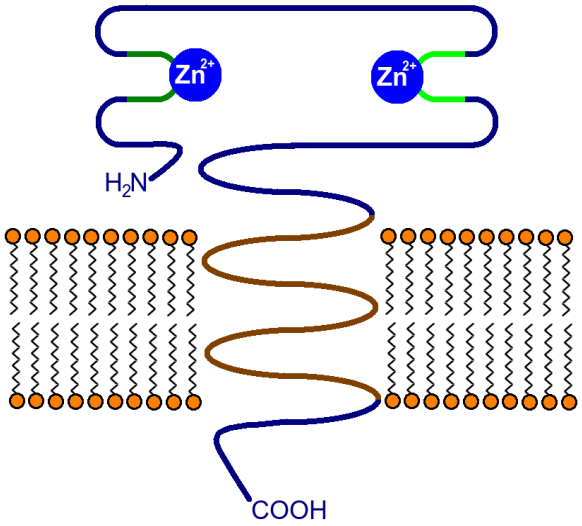
Might fail if
- big conformational changes at receptor site, depending on the ligand
- docking and scoring not accurate enough

Structure-Based Design – Case Study

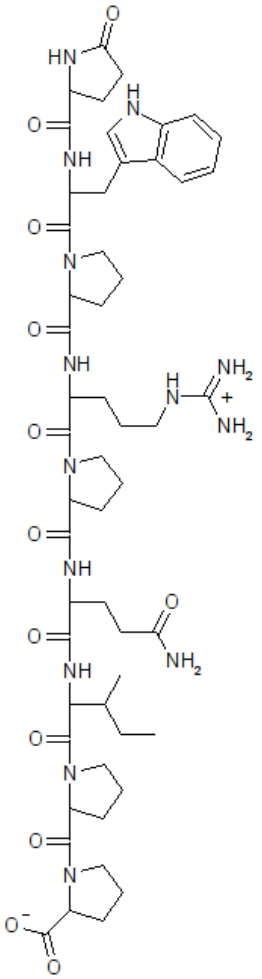
Domain-Selective ACE Inhibitors

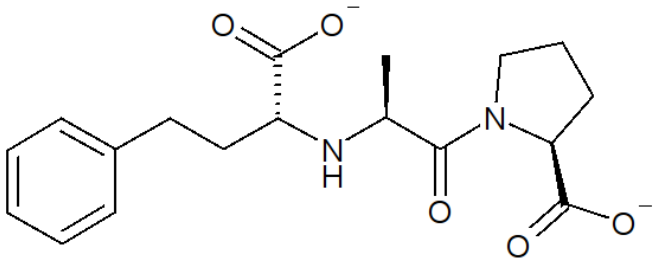


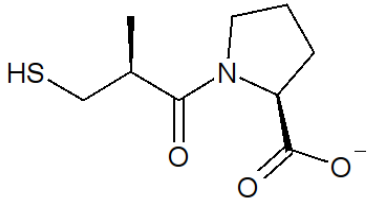
Ligand-Based Design of ACEI-s (pre-2003)



snake venom







Enalaprilat ***Captopril*** ***Teprotide***

Structure-Based Design

… applied when **3D structure** of the **target** protein **is known.**

Crystal structure of the human angiotensin-converting enzyme–lisinopril complex

Ramanathan Natesh*, Sylva L. U. Schwager†, Edward D. Sturrock† & K. Ravi Acharya*

** Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK*
† Division of Medical Biochemistry and MRC/UCT Liver Research Centre, University of Cape Town Medical School, Observatory 7925, South Africa

Nature (2003), 421: 551-554.

Structure-Based Design

… applied when **3D structure** of the **target** protein **is known.**

Crystal Structure of the N Domain of Human Somatic Angiotensin I-converting Enzyme Provides a Structural Basis for Domain-specific Inhibitor Design

Hazel R. Corradi[1], Sylva L. U. Schwager[2], Aloysius T. Nchinda[2] Edward D. Sturrock[2]* and K. Ravi Acharya[1]*

[1]*Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK*

[2]*Division of Medical Biochemistry and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Observatory 7925 South Africa*

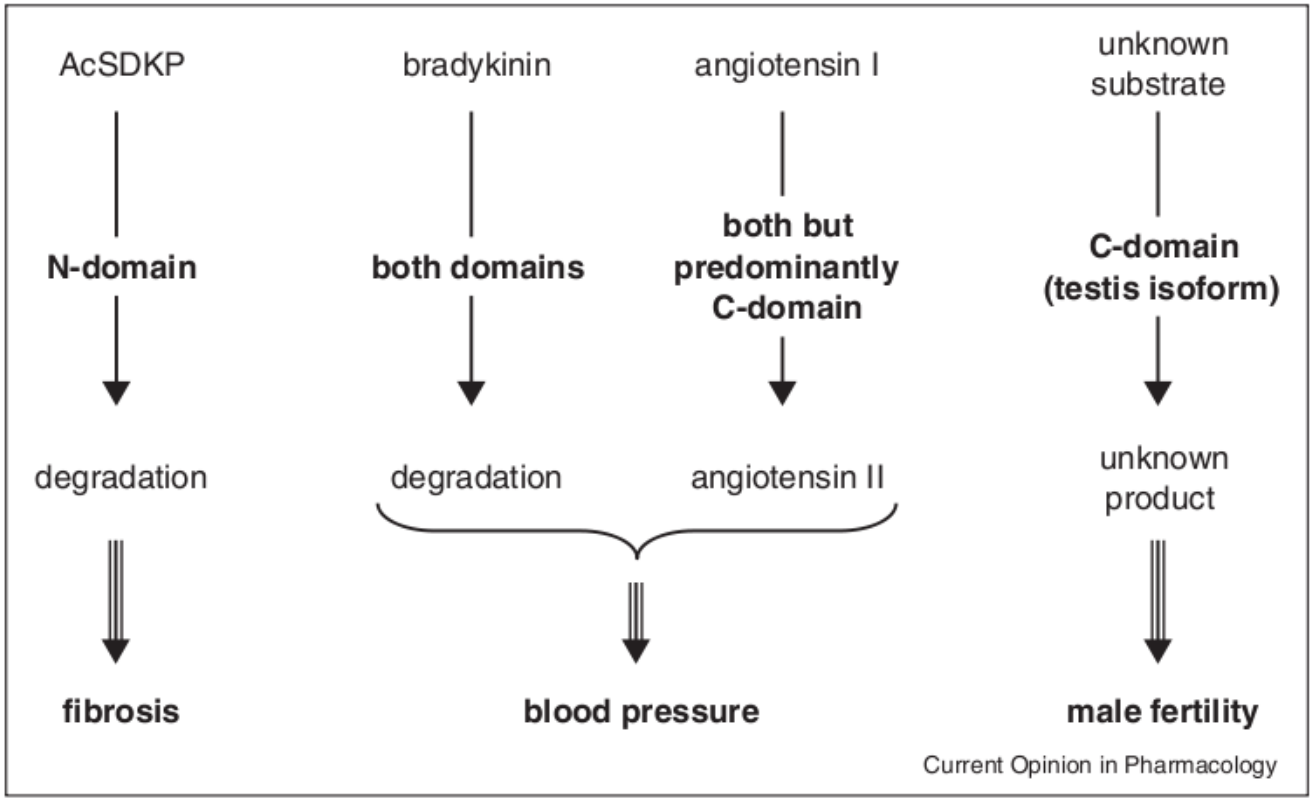
**Corresponding author*

Human somatic angiotensin I-converting enzyme (sACE) is a key regulator of blood pressure and an important drug target for combating cardiovascular and renal disease. sACE comprises two homologous metallopeptidase domains, N and C, joined by an inter-domain linker. Both domains are capable of cleaving the two hemoregulatory peptides angiotensin I and bradykinin, but differ in their affinities for a range of other substrates and inhibitors. Previously we determined the structure of testis ACE (C domain); here we present the crystal structure of the N domain of sACE (both in the presence and absence of the antihypertensive drug lisinopril) in order to aid the understanding of how these two domains differ in specificity and function. In addition, the structure of most of the inter-domain linker allows us to propose relative domain positions for sACE that may contribute to the domain cooperativity. The structure now provides a platform for the design of "domain-specific" second-generation ACE inhibitors.

Keywords: angiotensin I-converting enzyme; cardiovascular disease; crystal structure; hypertension; inhibitor design

J. Mol. Biol. (2006), 357: 964–974.

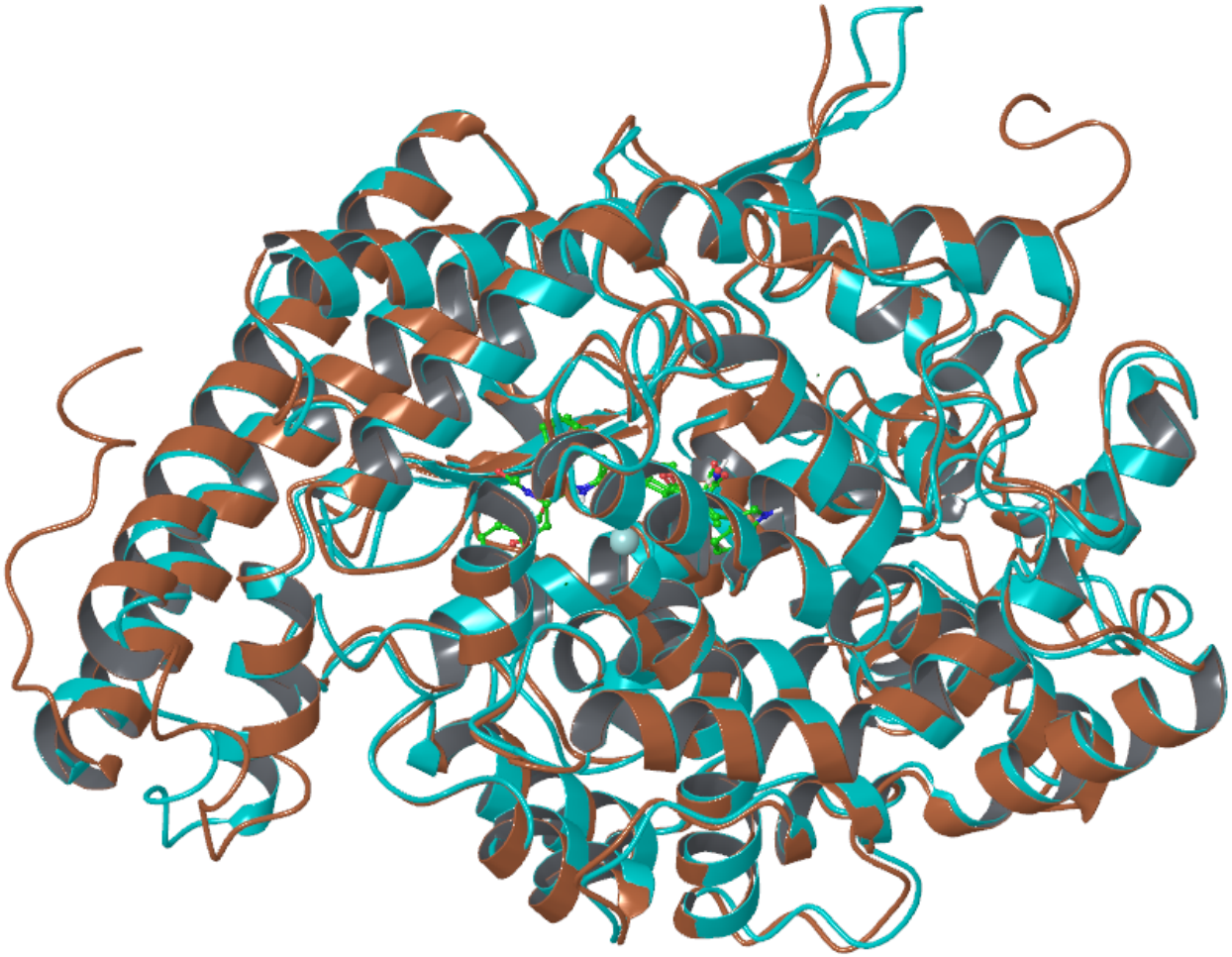
ACE domains *in vivo*



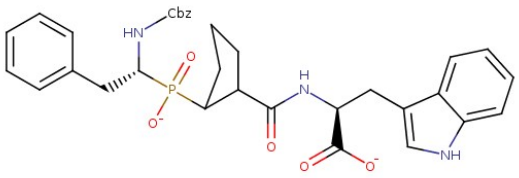
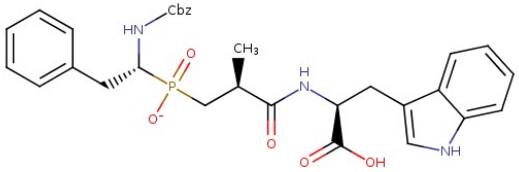
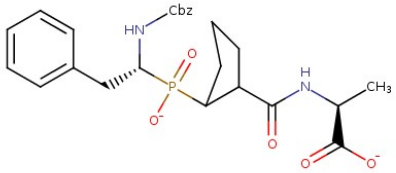
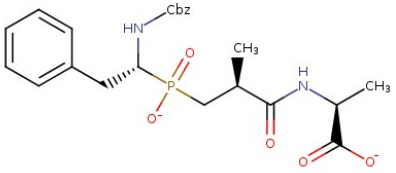
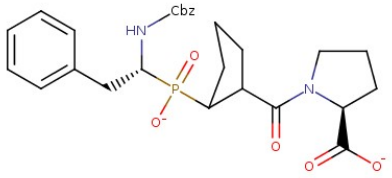
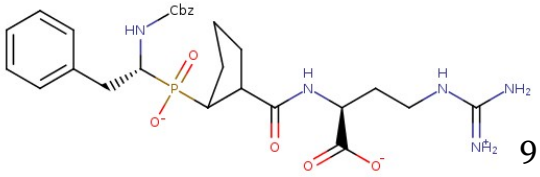
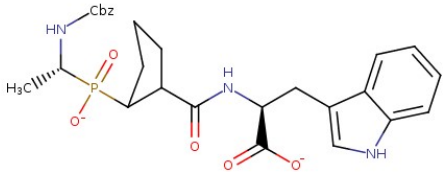
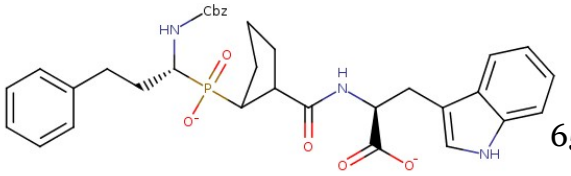
Current Opinion in Pharmacology (2011), 11:105–111.

ACE domains

Superposition of N-domain and C-domain



Domain-selective ACEI-s: structure – activity relationship

	$K_i(C)$ [nM]	$K_i(N)$	selectivity index
	3	10 000	3 300
	0.5	45	90
	20	450	22
	0.8	0.8	1
	4	60	15
	9	200	22
	60	8000	130
	65	9 000	138

Biochemistry (2004), 43: 8048-8054

Domain-selective ACEI-s: predicted binding mode

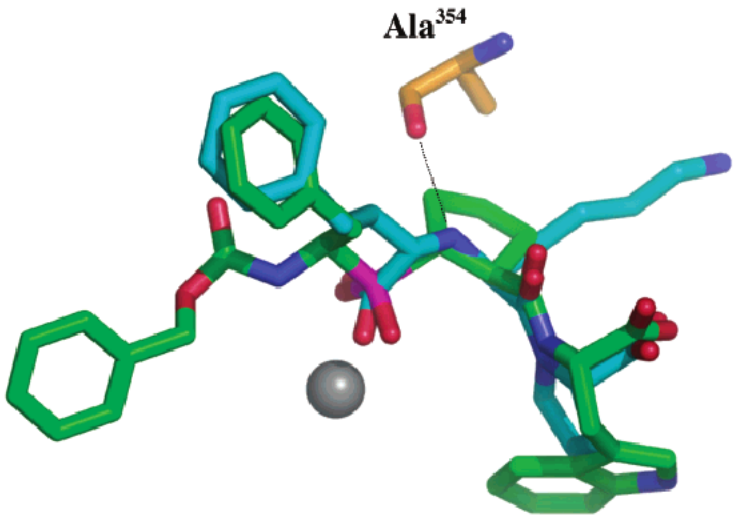
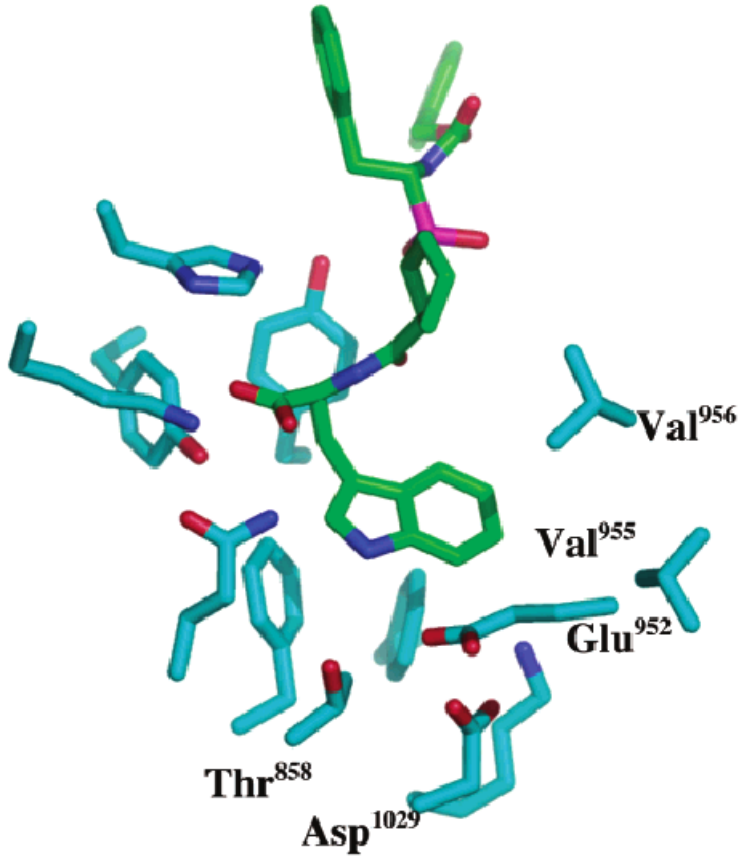


Table 2: Comparison of the Residues between the N- and C-Domains of Somatic Human ACE Delineating the S_2' Subsite

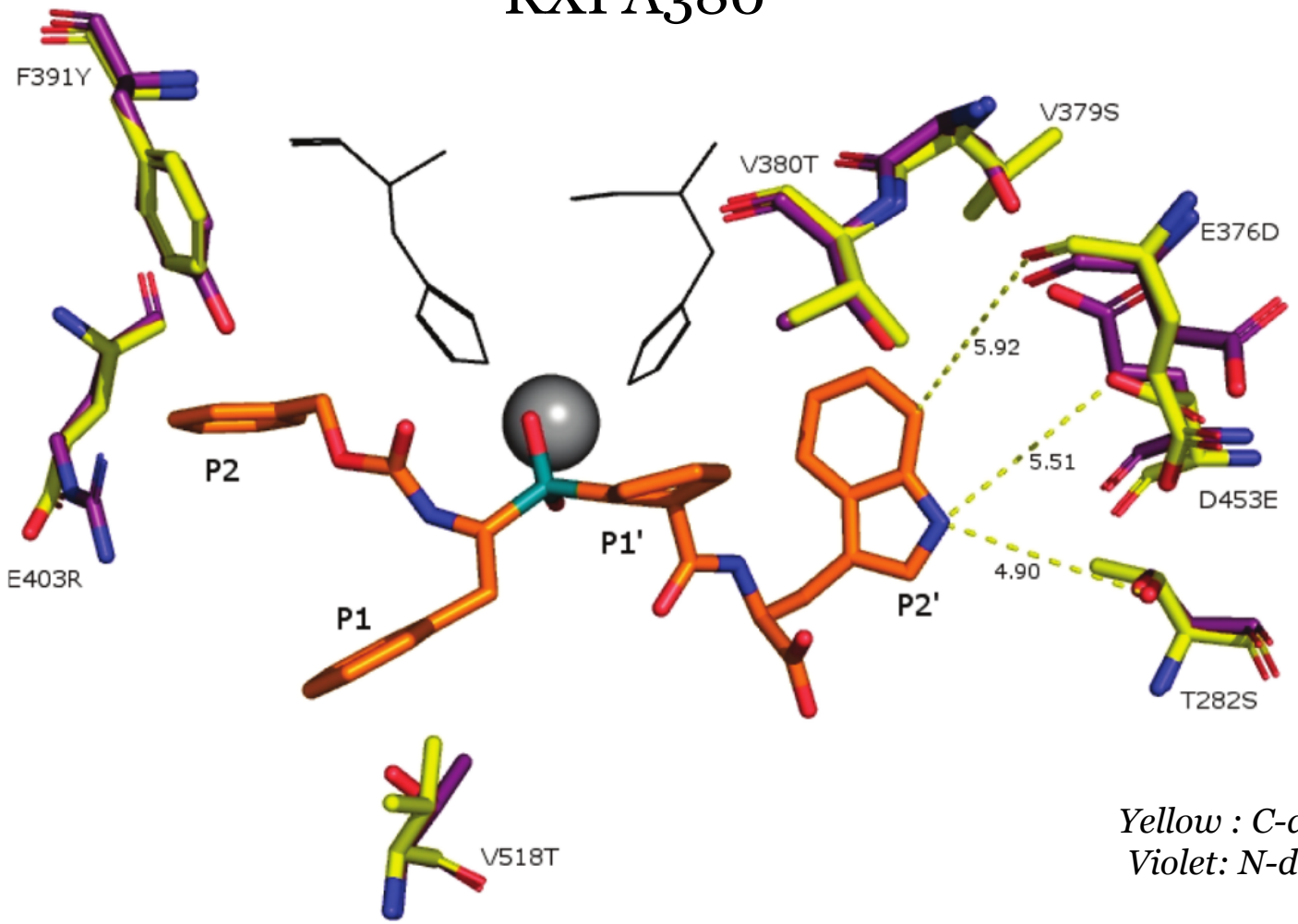
N-domain	C-domain	N-domain	C-domain	N-domain	C-domain
Gln 259	Gln 857	Thr 358	Val 956	Phe 438	Phe 1036
Ser 260	Thr 858	Asp 393	Asp 991	Tyr 498	Tyr 1096
Asp 354	Glu 952	Glu 431	Asp 1029	Tyr 501	Tyr 1099
Ser 357	Val 955	Phe 435	Phe 1033	Phe 505	Phe 1103



Biochemistry (2004), 43: 8048-8054

Domain-selective ACEI-s

RXPA380

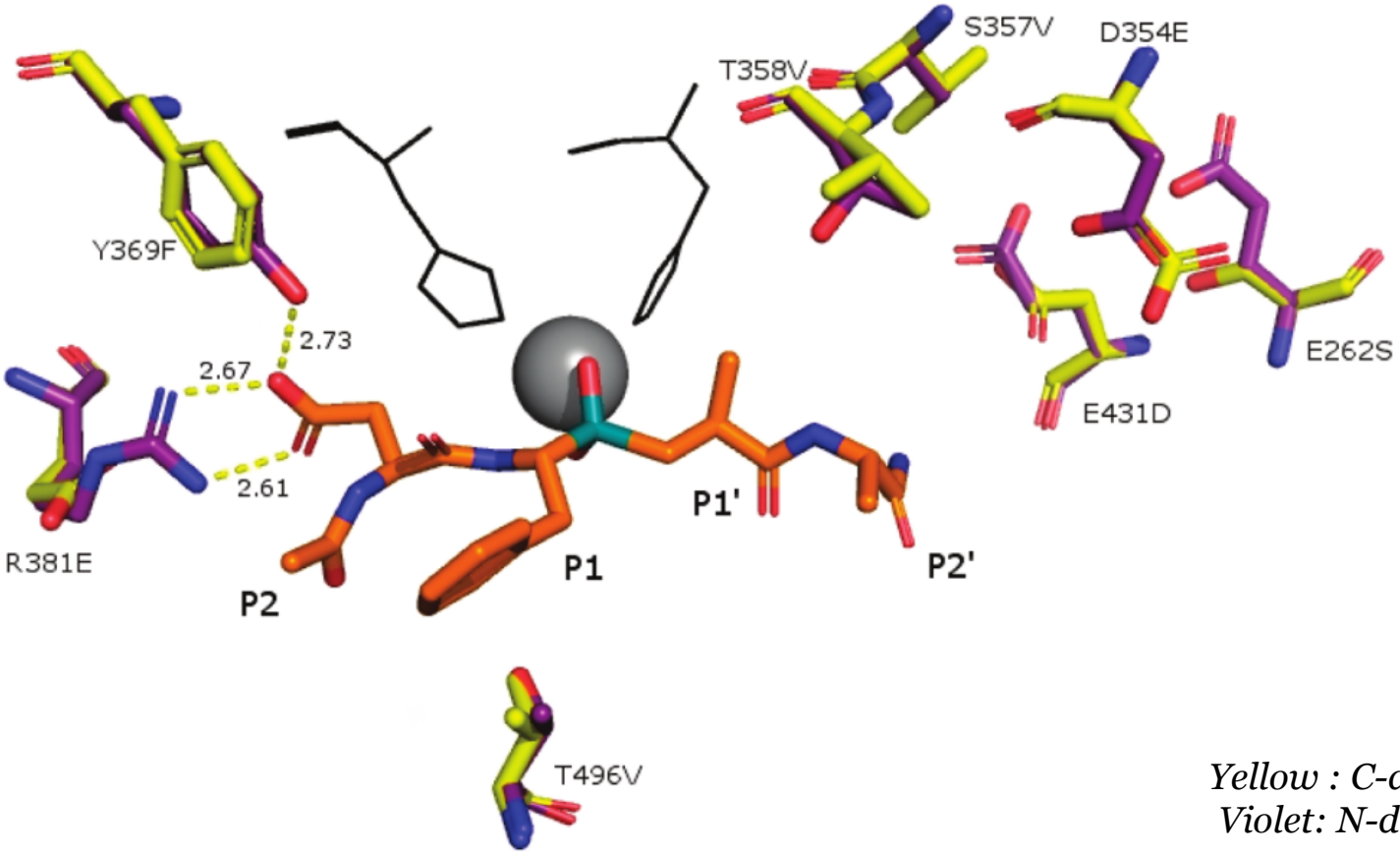


Yellow : C-domain
Violet: N-domain

Biochemistry (2009), 48: 8409-8412

Domain-selective ACEI-s

N-domain selective: RXP407

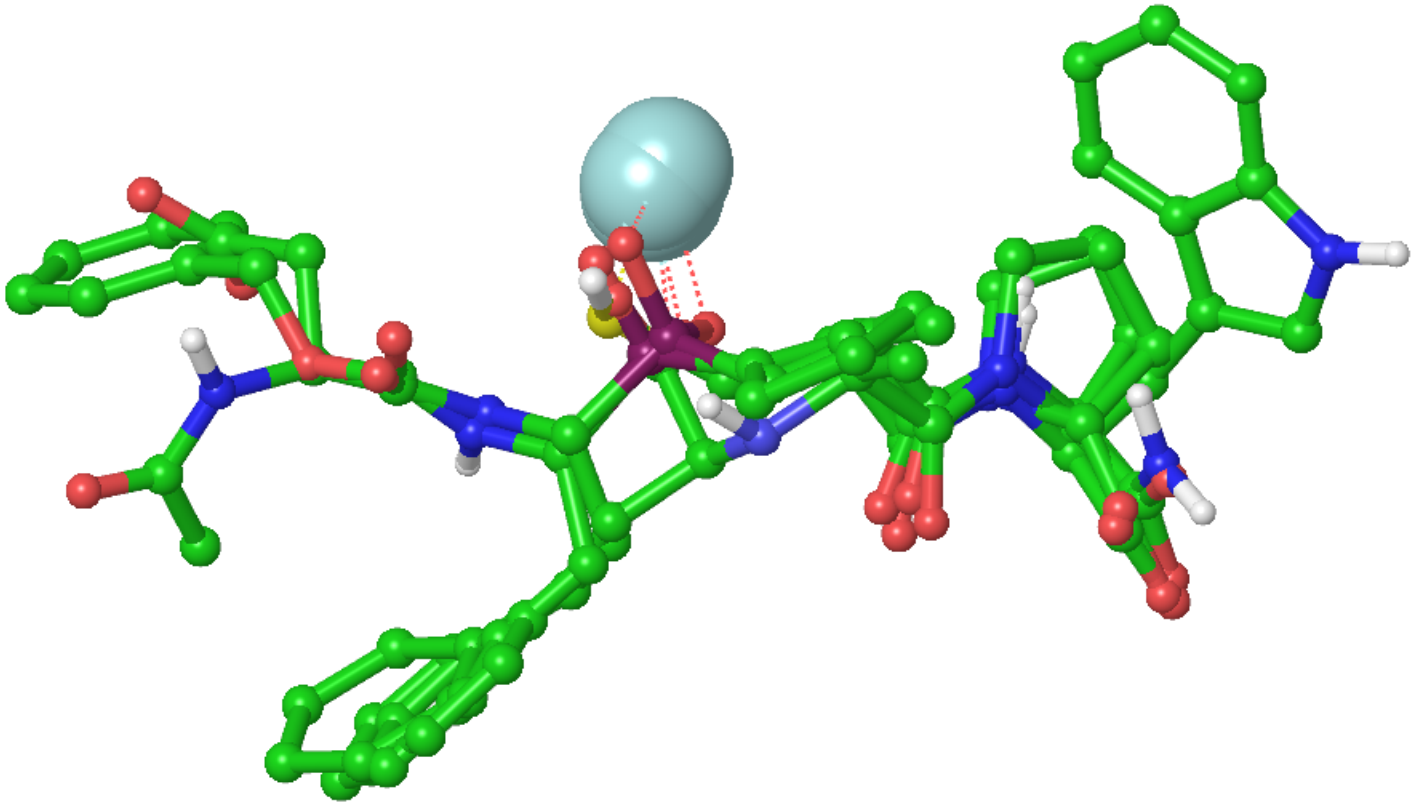


Yellow : C-domain
Violet: N-domain

Biochemistry (2009), 48: 8409-8412

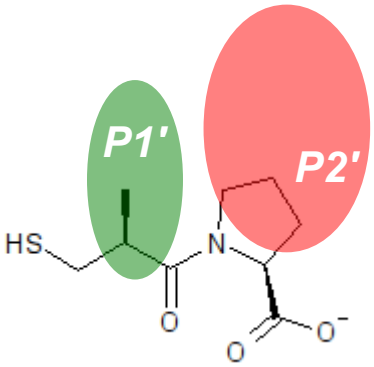
Superposition of crystal poses of ACEI-s

PDB ID (year published) : 1UZE (2004), 1UZF (2004), 2OC2 (2010), 3NXQ (2010)

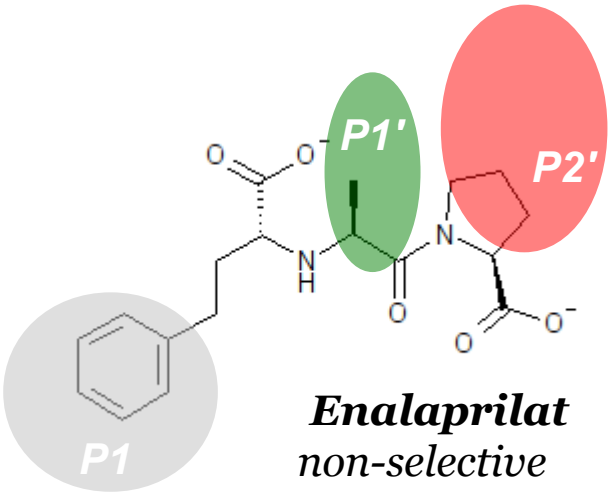


J.Biol.Chem. (2010), 46: 5473-5478

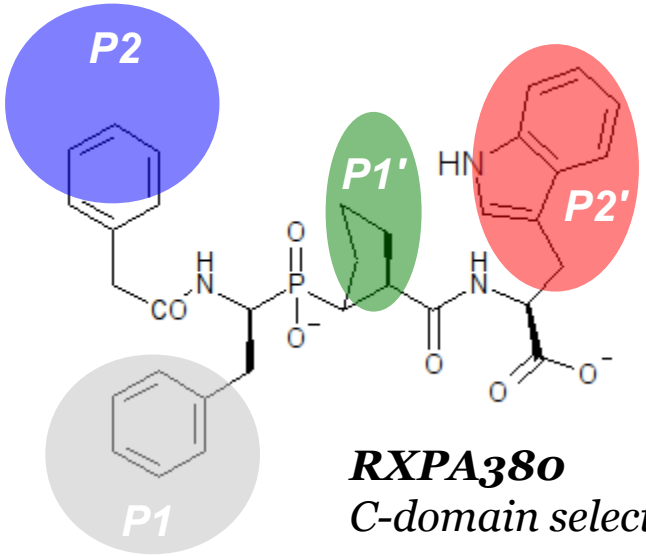
SBD of domain-selective ACEI-s



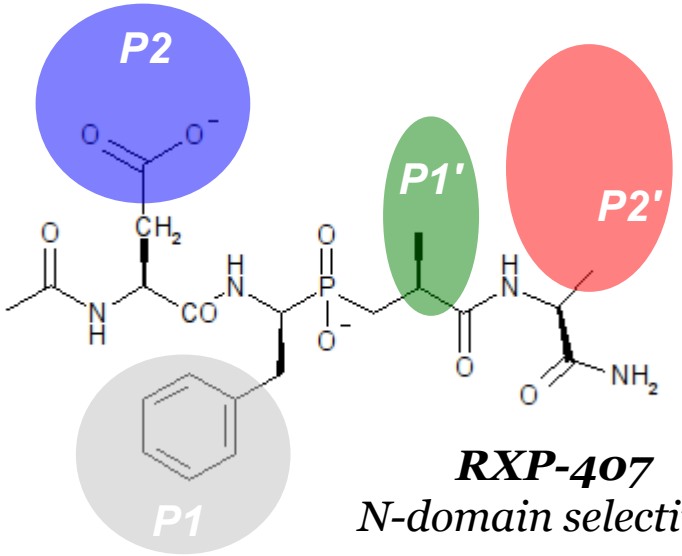
Captopril
non-selective



Enalaprilat
non-selective



RXPA380
C-domain selective



RXP-407
N-domain selective

Knowing the 3D structure of both active sites

- fully utilize structure-based design methods in order to exploit differences in the binding sites of the two domains → maximize sub-site specific interactions

- the most advanced inhibitors can distinguish between two catalytic domains with a selectivity factor > 1 000 thanks to:

  C-domain → hydrophobic interactions in S_2 and S_2'

  N-domain → salt-bridge in S_2

- extending molecule of the first ACE inhibitor ***Captopril*** resulted in several new favorable interactions with the enzyme binding pockets improving selectivity toward ACE in general

- rigidification (S_1'), optimizing charge distribution, improve affinity by displacing solvent from the sub-sites