

SYSTEMATIC SEARCH AND PHARMACOPHORES

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The Pharmacophore Concept (Active-site Mapping)

**MOST THERAPEUTIC TRAGETS ARE MACROMOLECULES
WITH UNKNOWN 3D STRUCTURES**

**OFTEN, THE STRUCTURE OF A DIVERSE SET OF
COMPOUNDS THAT BIND TO THE THERAPEUTIC TARGET
ARE KNOWN THROUGH COMBINATORIAL SYNTHESIS OF
COMPOUND LIBRARIES AND HIGH-THROUGHPUT
SCREENING**

**IF THE ACTIVE COMPOUNDS HAVE SUFFICIENTLY DIVERSE
STRUCTURES, THEN ONE CAN USE THE COMPUTER TO
SEARCH FOR COMMON SURFACES THAT THE RECEPTOR
MIGHT RECOGNIZE
(ACTIVE ANALOG APPROACH)**

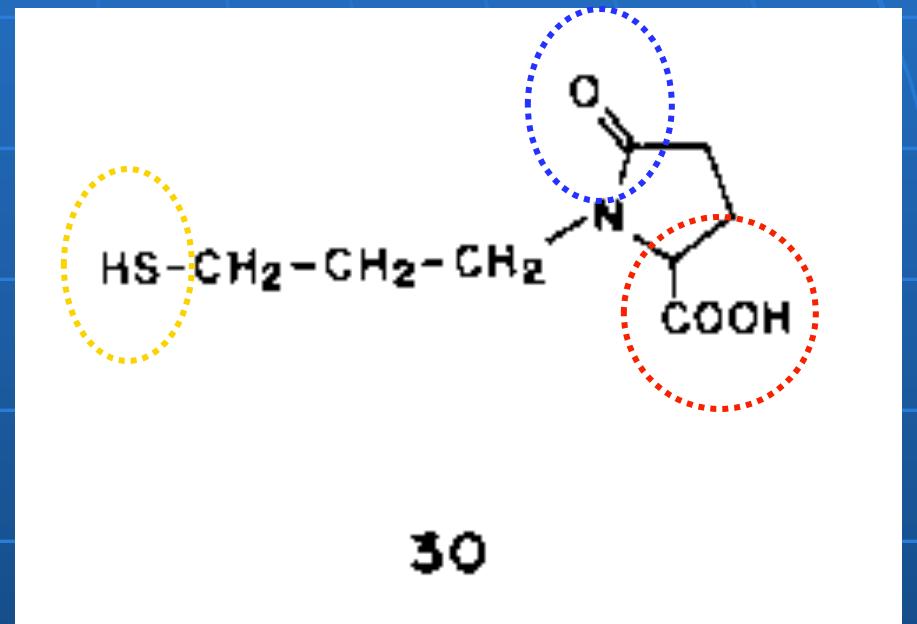
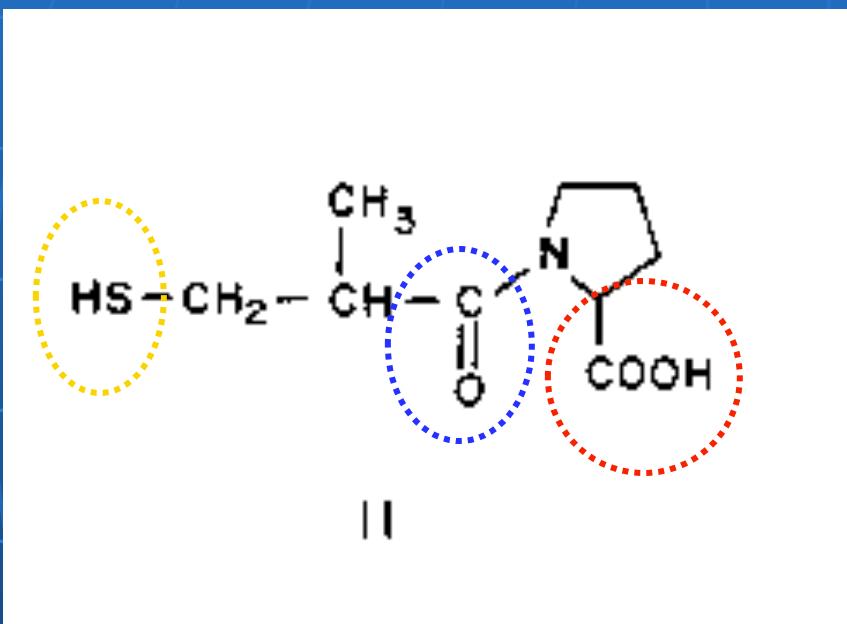
Interactivity is expected!

- **Easy fundamentals—just need to connect them**
- **If a concept is unclear, ask immediately!**
- **After each major concept will be a “question slide”**
 - **Someone *must* ask a question**
 - **Another in the class must answer this question**
- **Each slide is numbered so you can reference**

Things to know, 90 minutes from now

- **What is a pharmacophore?**
 - Pattern recognition
 - Distance constraints
 - Degrees of freedom and conformational hyperspace, systematic search algorithms
- **Quantitative structure-activity relationships (QSAR, COMFA, PCA and PLS)**
- **How to derive constraints from physical interactions between ligand and receptor?**
- **How to apply these constraints to predict active 3D conformations of bound ligands?**
- **How to identify a good (or bad) prediction?**

Quiz: which of these is active?



- How to judge whether or not a molecule might be active, based only on structural info?

Pharmacophore - Definition

- **Paul Erlich, early 1900**

“a molecular framework that carries (*phoros*) the essential features responsible for a drug’s (*pharmacon*) biological activity”

- **Peter Gund, 1977**

“a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule’s activity”

- **Wikipedia, today**

A pharmacophore is a 3-dimensional substructure of a molecule that carries (*phoros*) the essential features responsible for a drug’s (*pharmacon*) biological activity. Alternatively described as an ensemble of interactive functional groups with a defined geometry. Basically, one tries to talk the protein language by finding the “structural and chemical complementaries” (aka pharmacophore hypothesis) to target receptors

Pharmacophore modeling

■ Analog-based pharmacophores

- Unknown receptor
- Information from active and inactive molecules
- 3D-QSAR

■ Receptor-based pharmacophores

- Hypothetical receptor site based on function (enzyme)
- Known (and characterized) receptor
- Docking

Quantitative Structure-Activity Relationships (QSAR)

- **Activity = function(structural/chemical properties)**
- **Example***
 - **Relate biological activity to electronics and hydrophobicity**
 - $\log(1/C) = k_1 \log P - k_2 (\log P)^2 + k_3 s + k_4$
 - C: concentration of compound that gives a response
 - P: partition coefficient between water and 1-octanol
 - k_1, k_2, k_3, k_4 : constants
 - s: Hammett substituents parameter

*[C.Hansch. (1969) Accounts of Chemical Research. 2:232-239]

QSAR process

- **Synthesize & test biological activity for diverse set of ligands (including actives and inactives)**
- **Clever experimental design maximizes information content from a series**
- **Regression techniques to fit an equation to the data**
- **Which properties are correlated with activity?**
- **Cross-validate**
- **Predict, wary of extrapolation vs. interpolation**

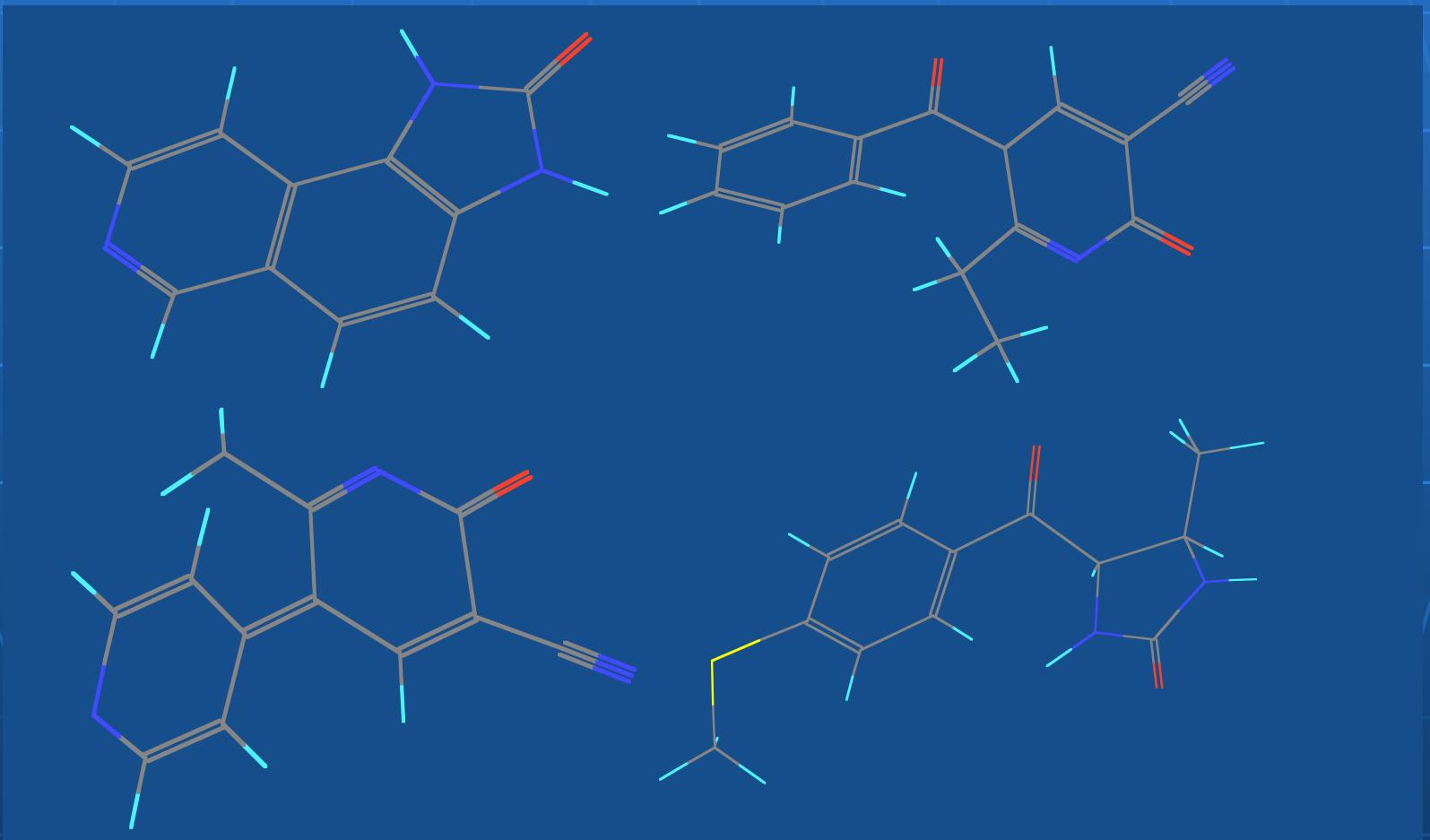


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QSAR Objective

- **Correlate 3D structure of a ligand with its biological activity**
- **Problems**
 - **Frame of reference**
 - **Unknown steric interactions**
 - **Multiple binding modes**
 - **Underdetermined system**

A simple example

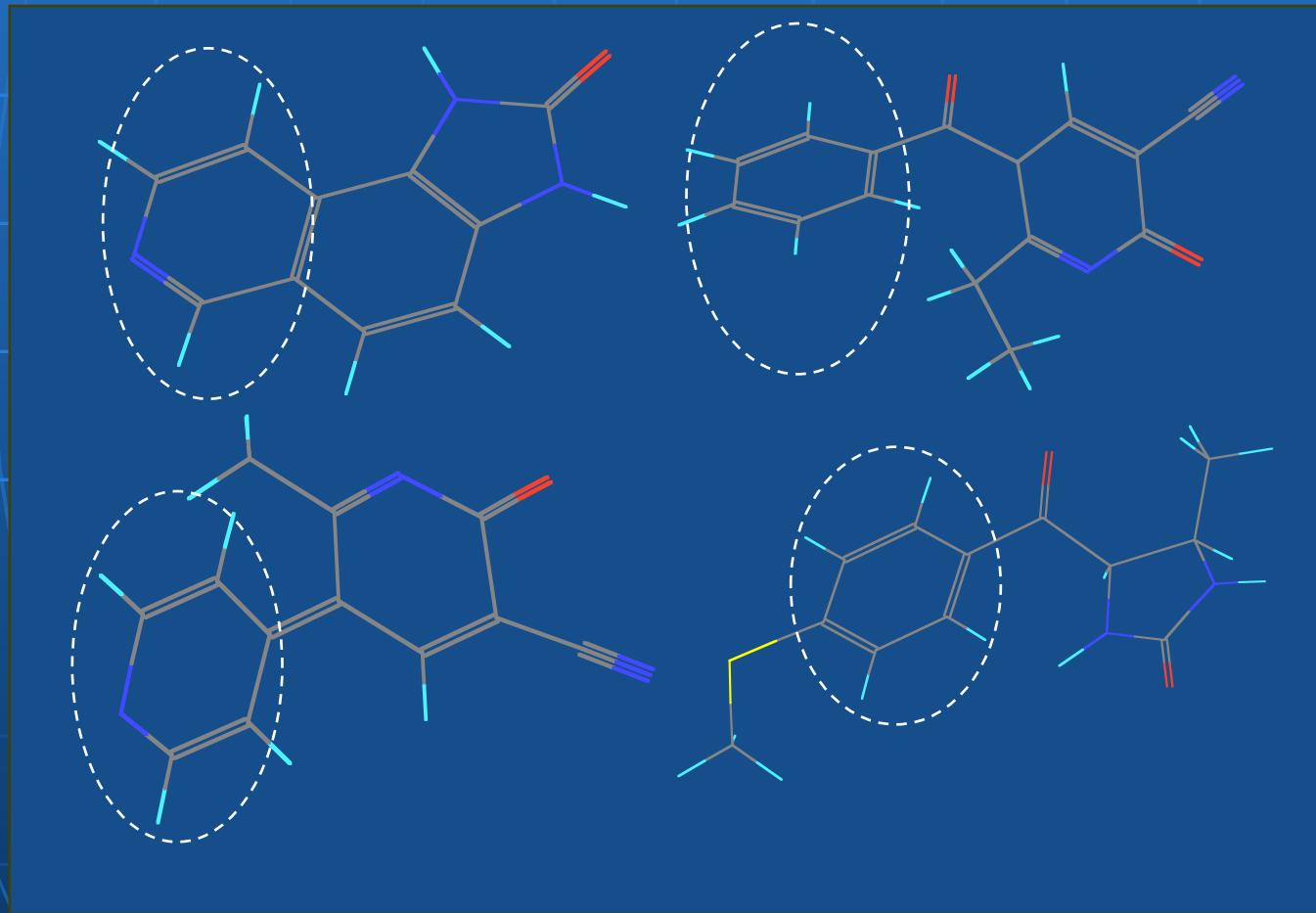


Visual Pattern Recognition

- ❖ **Visual identification of common structural and chemical features among active molecules and those features that are missing in the inactive ones**
- ❖ **Measurement of the 3D aspects of the common features, w.r.t. each other**
- ❖ **Development of a draft pharmacophore**
- ❖ **Validation that the pharmacophore fits the active compounds and fails to fit the inactive ones**
- ❖ **Refinement of the model by applying it to a database of compounds with known activity, until the desired result is reproduced**

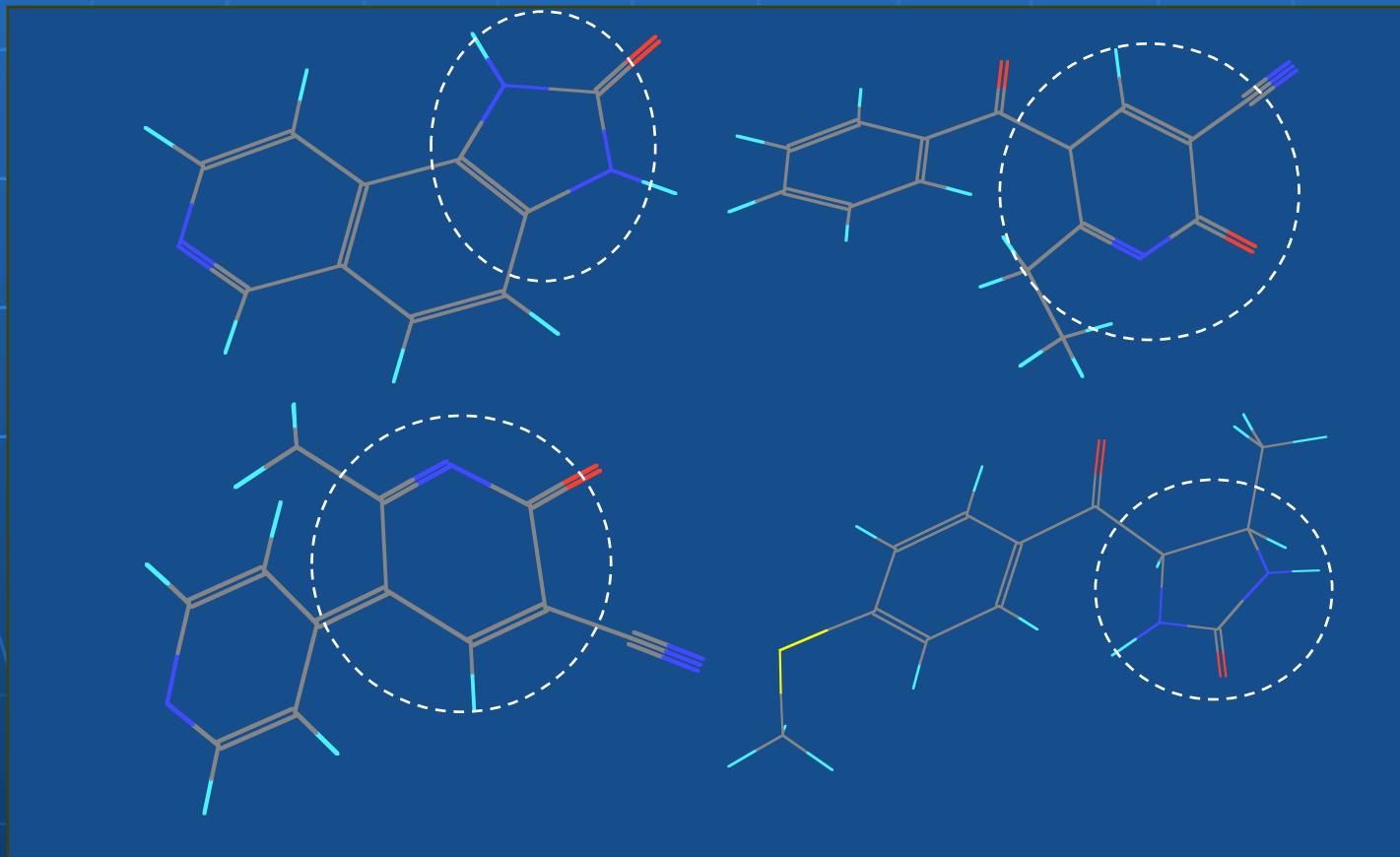
Visual Pattern Recognition

One aromatic ring: phenyl or pyridyl



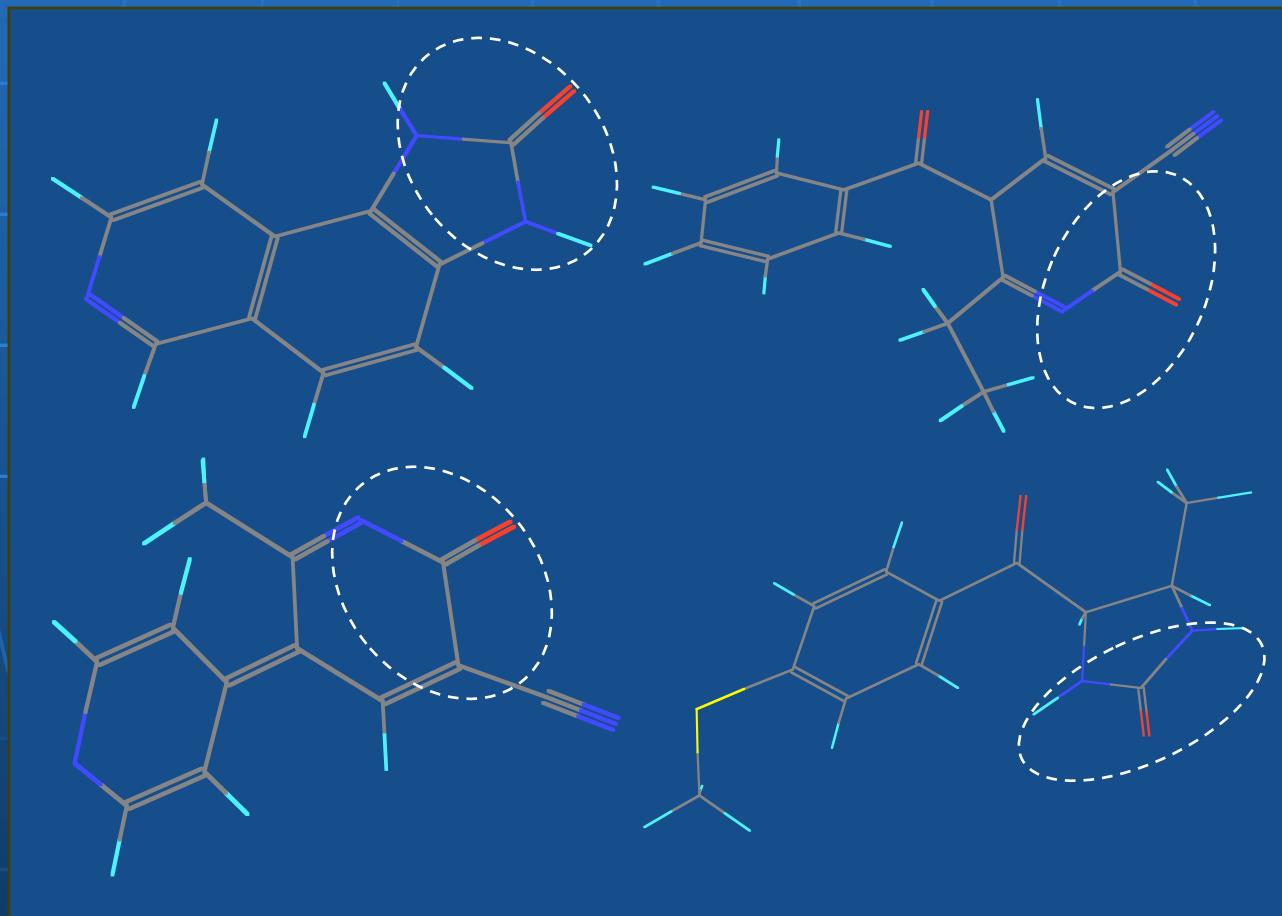
Visual Pattern Recognition

Second 5- or 6-membered ring



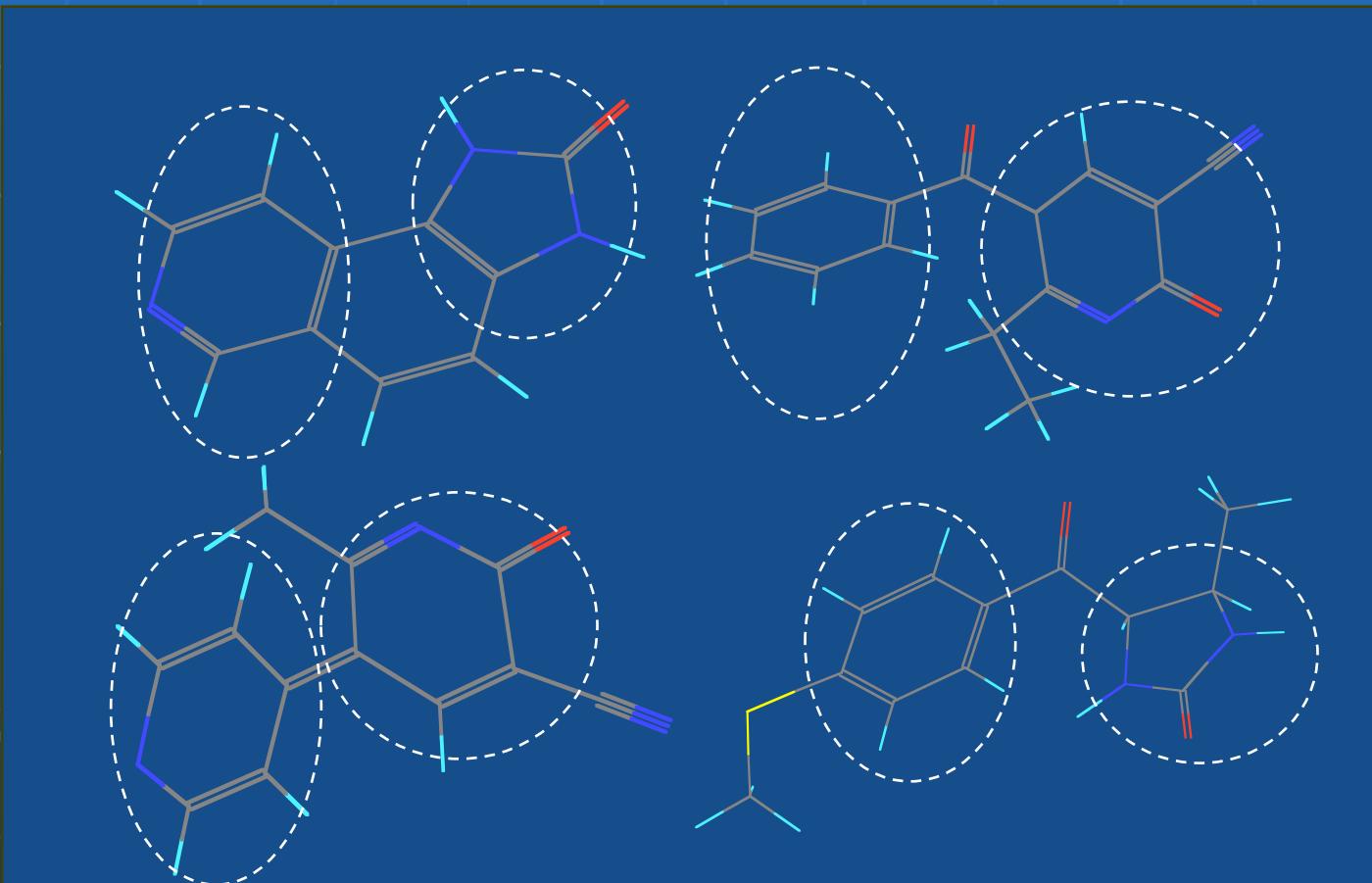
Visual Pattern Recognition

Urea group or amide functionality in 2nd ring



Visual Pattern Recognition

Ring systems are side-by-side

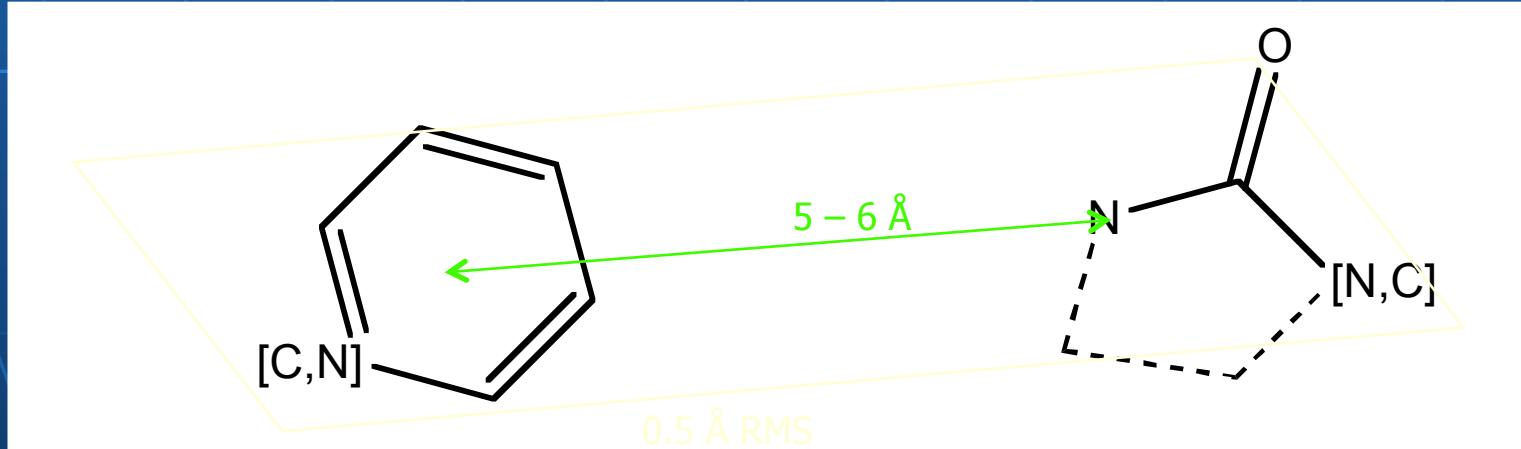


Measurements

- From the amide nitrogen to the center of the aromatic ring is approx 5-6 Å
- The aromatic ring and amide group are within 0.5 Å RMSD from planarity

Developing a pharmacophore model

- Phenyl or pyridyl ring
- 5- or 6-member ring with link option
- Additional nitrogen, various bond types
- Distance and planarity constraints

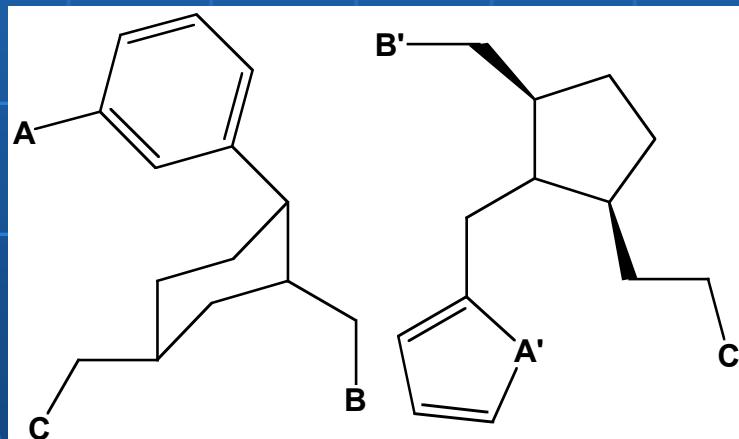




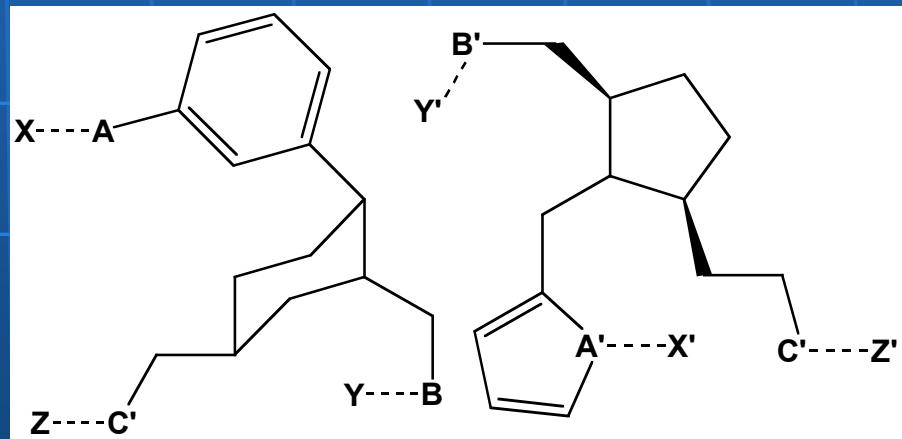
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Pharmacophore vs. Active Site Models

- Pharmacophore modeling with assumed ligand groups ($A=A'$, $B=B'$, $C=C'$)
- Active site modeling with receptor groups conceptually linked to ligand (X , Y , Z)



Active Analog



Active Site

Angiotensin Converting Enzyme (ACE): A useful 3D example

- **Historically important example**
 - **Evolution of rational drug design**
 - **Benchmark system for pharmacophore model**
- **Therapeutically relevant**
- **Clear illustration of balance between distance map resolution and sampling conformational hyperspace**

ACE “makes” Angiotensin II *in vivo*

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu
(C- terminus of Angiotensinogen)

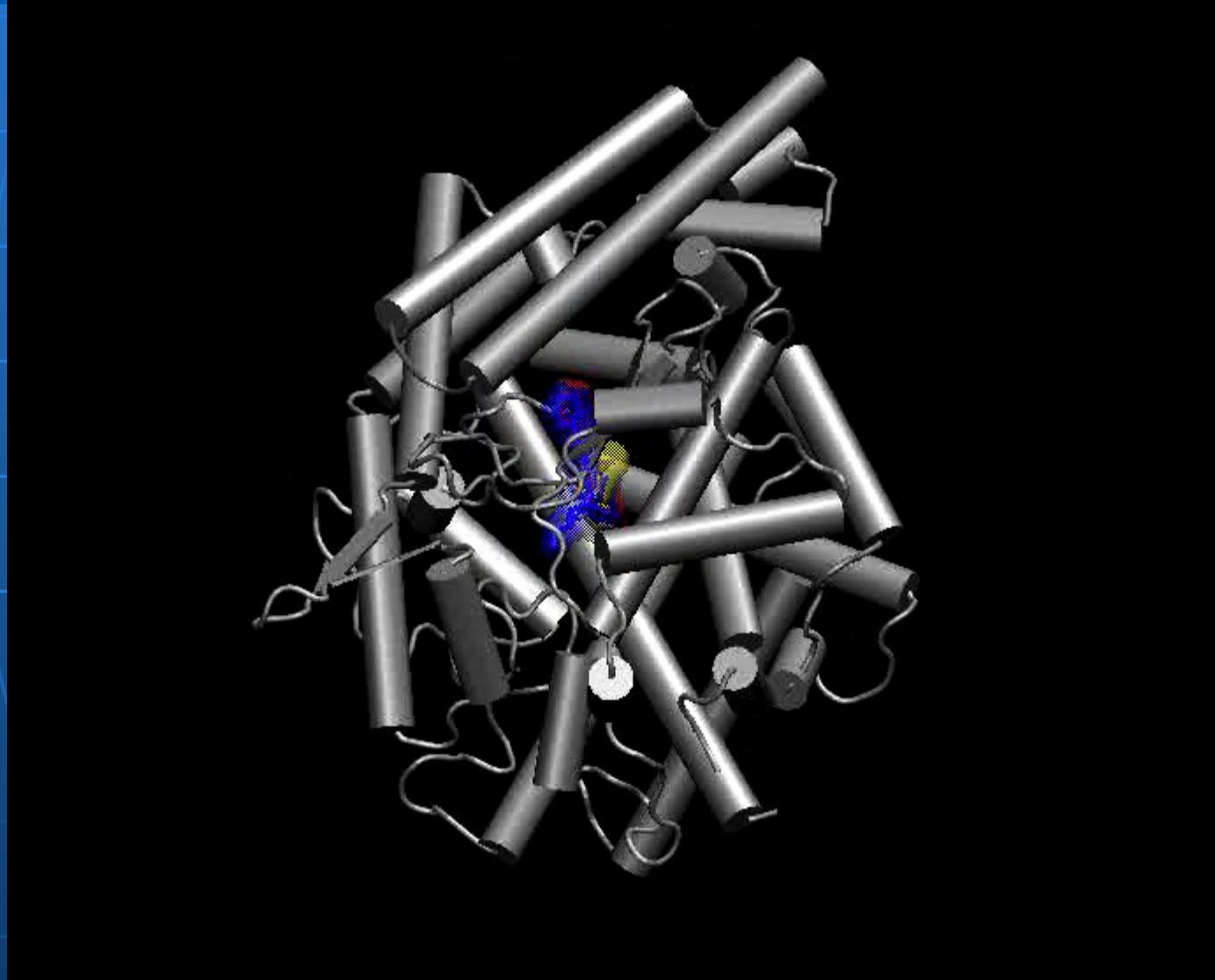
Renin

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
(Angiotensin I)

ACE

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
(Angiotensin II)

Background: the ACE system



Biological function of ACE

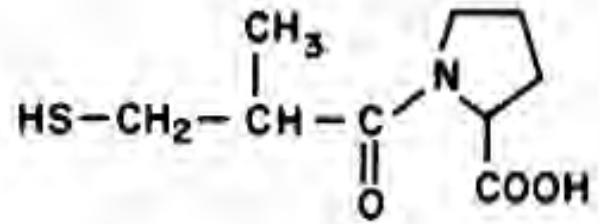
- **Overall pressor (pressure-raising) effect**
 - Converts decapeptide Angiotensin I to Angiotensin II
 - AII = potent vasoconstrictor
 - Stimulates release of steroid aldosterone
 - Increased sodium retention
 - Hydrolyzes C-terminal peptide from the hypotensive (vasodilator) nonapeptide bradykinin
 - Stimulates antidiuretic hormone (ADH) release
- **ACE action increases both vascular resistance and blood volume**
- **Inhibiting ACE results in antihypertensive effect**



How to Inhibit ACE?

- **Bradykinin-Potentiating Peptide (BPP)**
 - Naturally occurring nonapeptide in *Bothrops jararaca* venom
 - **BPP_{5a}** = pGlu-Lys-Phe-Ala-Pro
 - **Phe-Ala-Pro also inhibits ACE**
 - **Many studies to deduce structure-activity relationship with Phe-Ala-Pro mimics**
 - Complicated by pKa at titratable groups, etc
 - Simple computer models
 - **Several inhibitors emerge as potent**
 - **Good peptide-analog correlation for captopril**
 - **Poor peptide-analog correlation for enalapril**

Captopril

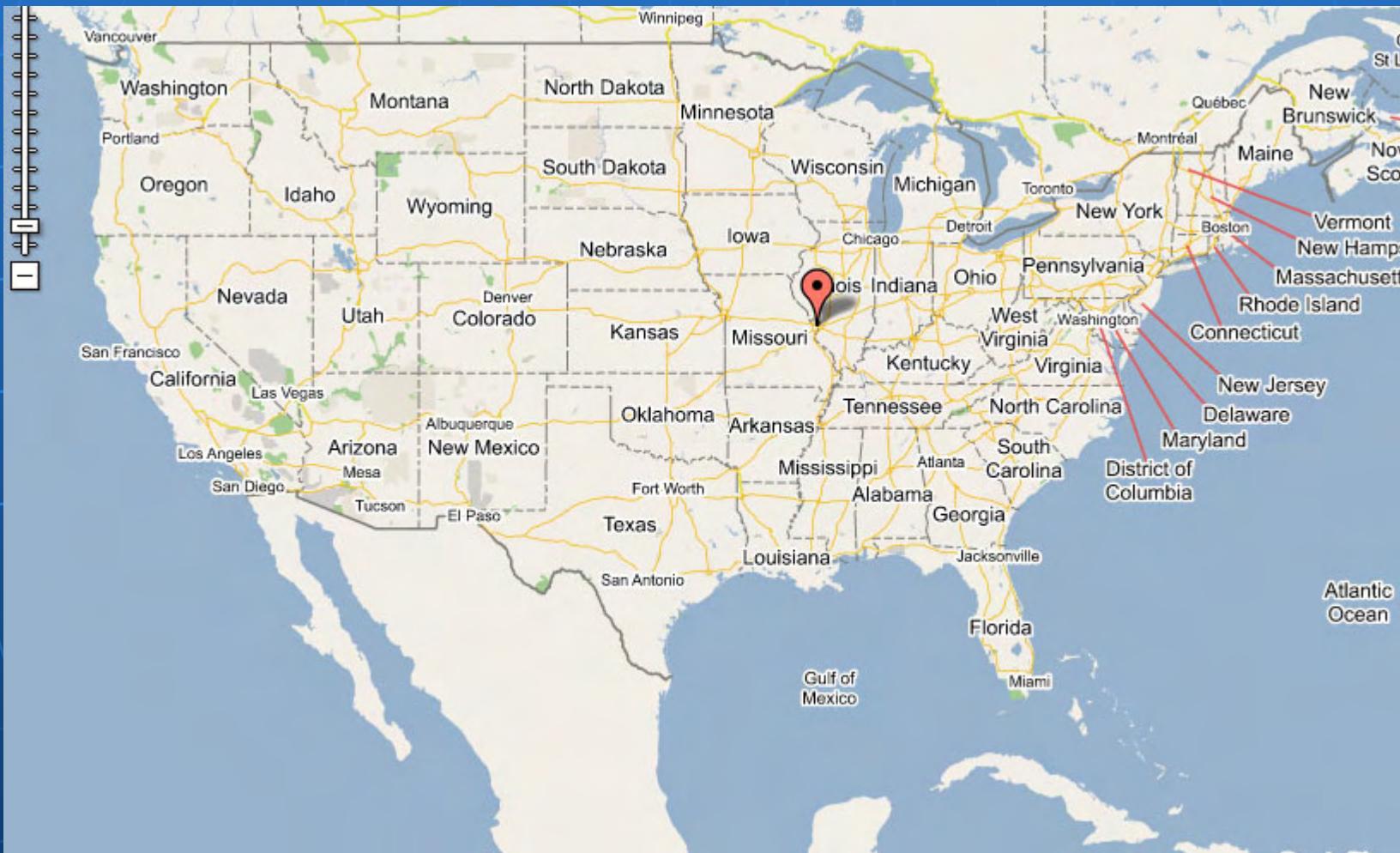


- **Designed based upon BPP_{5a}**
- **First ACE inhibitor drug**
 - **Orally available**
 - **Potent**
- **Undesirable clinical side effects**
 - **Loss of taste**
 - **Rashes**
- **At therapeutic doses, does not interact with nervous system and cause side effects**
- **Similar sulphydryl moiety as penicillamine, similar side effects**

How to accurately deduce an active conformation?

- **Apply the active analog hypothesis**
- **Choose a potent inhibitor ($IC_{50} < 50\text{nm}$)**
- **Scan systematically through all torsions**
 - **Generate many conformations**
 - **Reject those with improbably large energy (VdW criterion)**
 - **# of conformers = $(360/\text{angle})^N$ - rejected**
 - $N = \# \text{ of rotatable bonds}$
 - **Small angles or large # of bonds → Combinatorial explosion!**
- **Create distance map (DMAP) relating structural features relevant to binding site**

Molecule = United States Atomic geometry = Geography

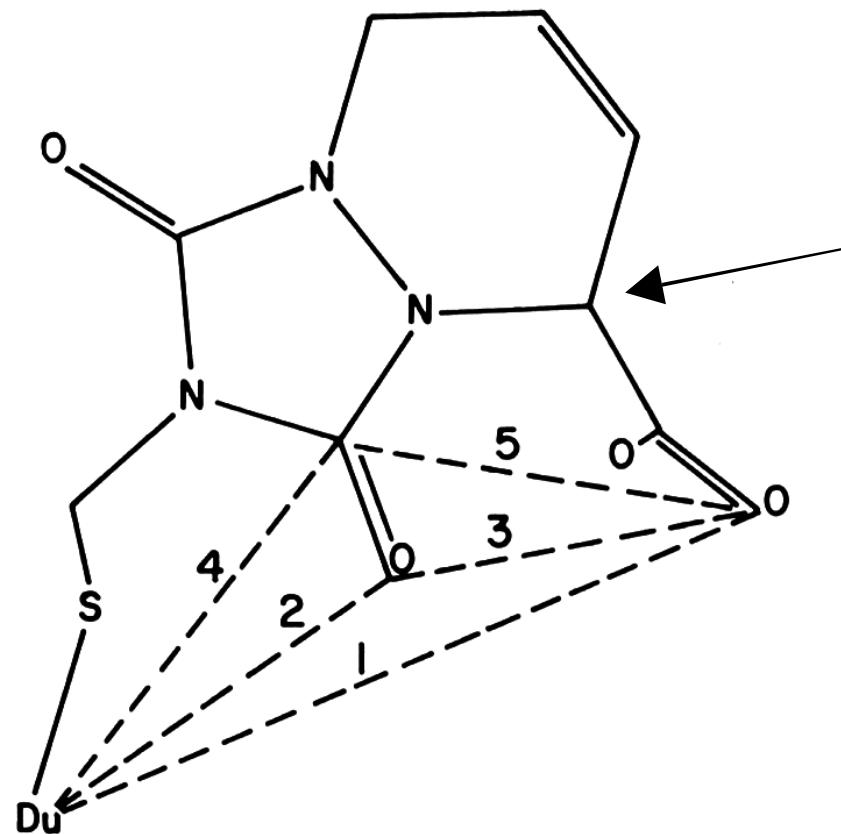


DMAP = Atlas driving distances

	Albuquerque NM	Atlanta GA	Baltimore MD	Billings MT	Birmingham AL	Boise ID	Boston MA	Buffalo NY	Charleston SC	Charleston WV	Charlotte NC	Cheyenne WY	Chicago IL	Cleveland OH	Columbia SC
Acadia N.P. ME	440	2459	1330	666	2468	1508	2958	274	733	1219	1039	1114	2221	1251	868
Albany NY		2041	1010	339	2098	1071	2518	169	301	880	636	772	1790	816	484
Albuquerque NM	2041		1404	1890	991	1254	940	2220	1773	1703	1600	1625	538	1312	1585
Atlanta GA	1010	1404		654	1799	148	2223	1108	907	291	501	240	1482	708	728
Baltimore MD	339	1890	654		1916	771	2406	427	365	568	362	418	1669	717	358
Big Bend N.P. TX	2239	588	1341	1907	1421	1192	1702	2297	1923	1623	1590	1583	966	1542	1714
Billings MT	2098	991	1799	1916		1775	606	2197	1721	2175	1721	1996	455	1231	1607
Birmingham AL	1071	1254	148	771	1775		2065	1226	902	441	561	391	1418	657	732
Boise ID	2518	940	2223	2406	606	2065		2685	2214	2493	2138	2345	734	1711	2026
Boston MA	169	2220	1108	427	2197	1226	2685		465	936	751	848	1923	994	657
Buffalo NY	301	1773	907	365	1755	902	2214	465		925	446	707	1498	539	191
Calgary AB	2434	1554	2369	2309	563	2256	794	2636	2148	2477	2100	2300	1019	1608	1964
Central Gavins N.P. NM	2078	209	1256	1873	1163	1113	1261	2224	1796	1585	1520	1496	604	1414	1599
Charleston SC	880	1703	291	568	2175	441	2493	936	925		478	210	1710	906	726
Charleston WV	636	1600	501	362	1721	561	2138	751	446	478		268	1405	470	248
Charlotte NC	772	1825	240	418	1996	391	2345	848	707	210	268		1615	737	516
Cheyenne WY	1790	538	1482	1669	455	1418	734	1923	1498	1710	1405	1615		981	1326
Chicago, IL	818	1312	708	717	1231	657	1711	994	539	906	470	737		981	348
Cincinnati, OH	748	1377	440	534	1522	461	1958	869	437	637	208	476	1190		287
Cleveland OH	484	1585	728	358	1607	732	2026	657	191	726	248	516	1326		348
Columbia SC	822	1623	214	517	2059	362	2385	955	801	113	362	94	1658	800	610
Columbus OH	637	1456	585	425	1608	574	1972	801	328	645	167	435	1297	358	140
Crater Lake N.P. OR	2926	1357	2632	2800	1023	2470	417	3099	2633	2856	2514	2744	1151	2111	2442
Dallas TX	1677	644	822	1357	1421	635	1593	1753	1364	1085	1049	1058	881	921	1189
Denver CO	1831	437	1430	1643	553	1325	835	1998	1553	1721	1370	1580	101	1021	1362

DMAP definition

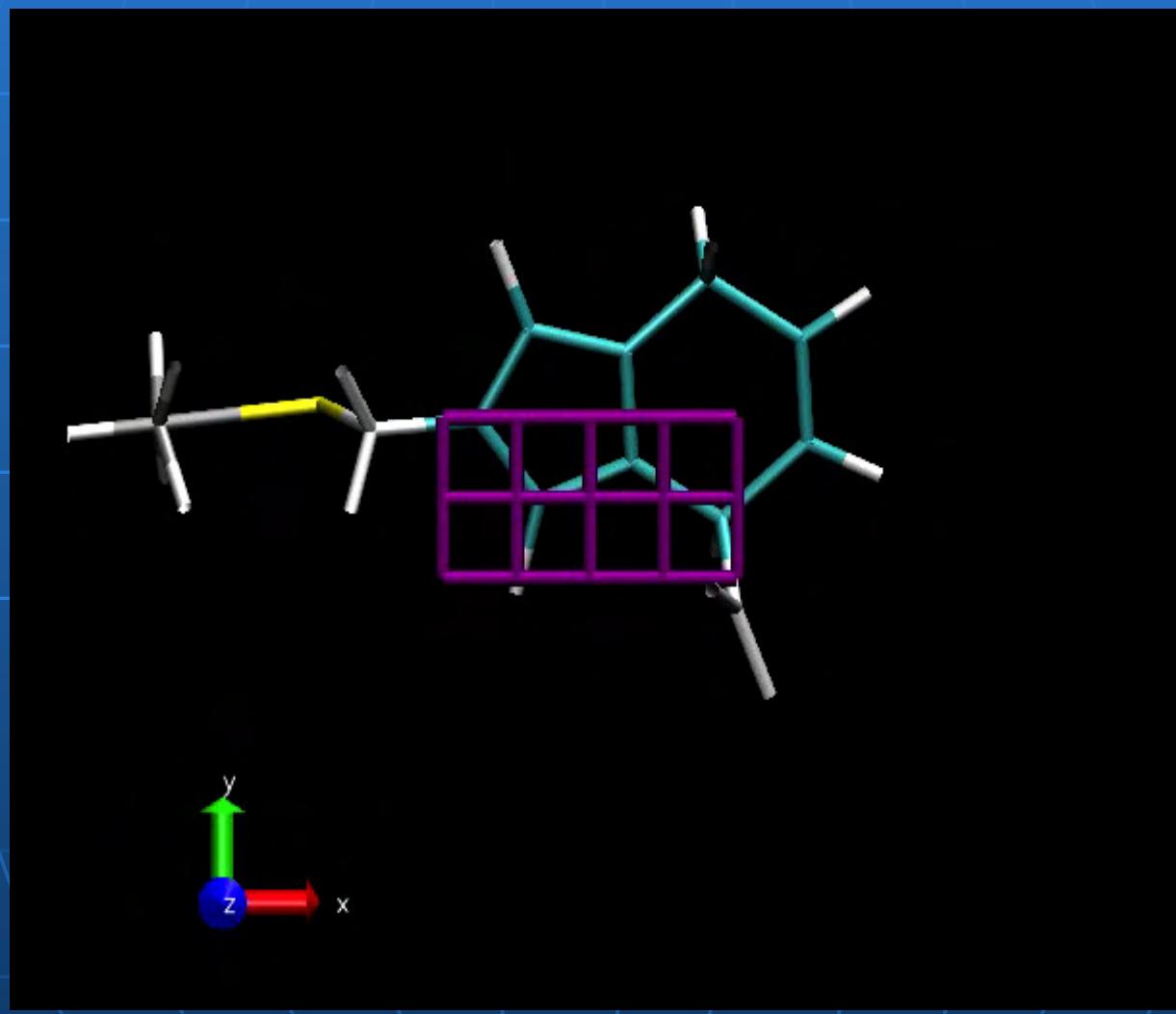
- $D_u = Z_n$
- DMAP valid for all active analogs
 - Z_n
 - $C=O$
 - $COOH$
- DMAP can become many-dimensional for some systems



Grid definition

- **How know if two DMAPs are equal?**
 - **Define a 3D grid around molecule**
 - **Two conformers evaluate to same grid points?**
 - **Effective resolution of predicted DMAP**
 - **~0.1-0.5 angstrom**
 - **Fine grid rejects more conformations**
- **Balance angle resolution with grid spacing!**

Can this molecule place the right atoms in the necessary volume?

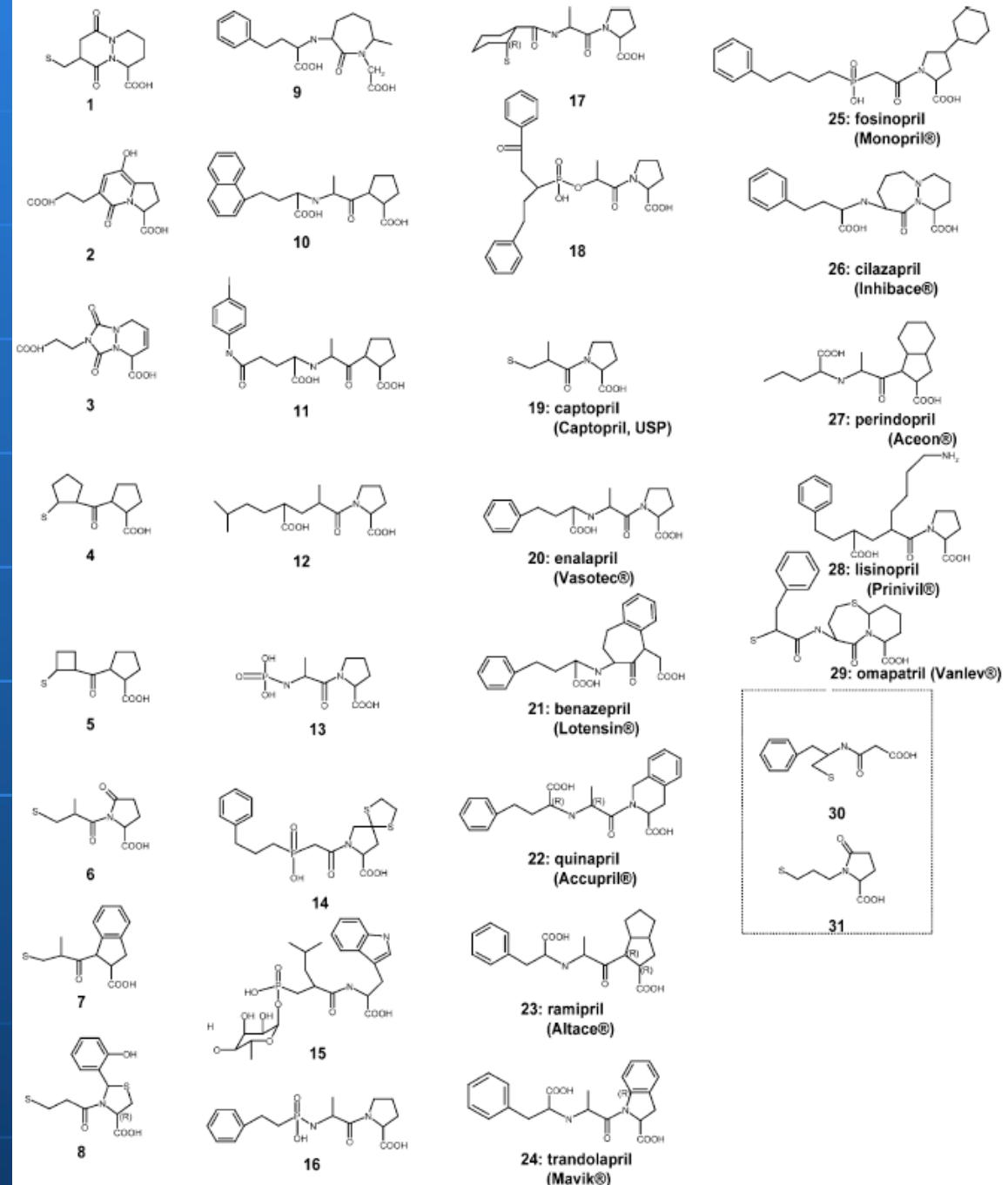




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ACE inhibitors generated by pharmaceutical industry to provide IP position for drug development.

2D chemical sketches of potent ACE inhibitors (#1–29) and two inactive compounds used as negative controls (#30–31). All chiral centers are in the S configuration unless explicitly noted here as R configuration. Compounds used in clinics have generic and trade names



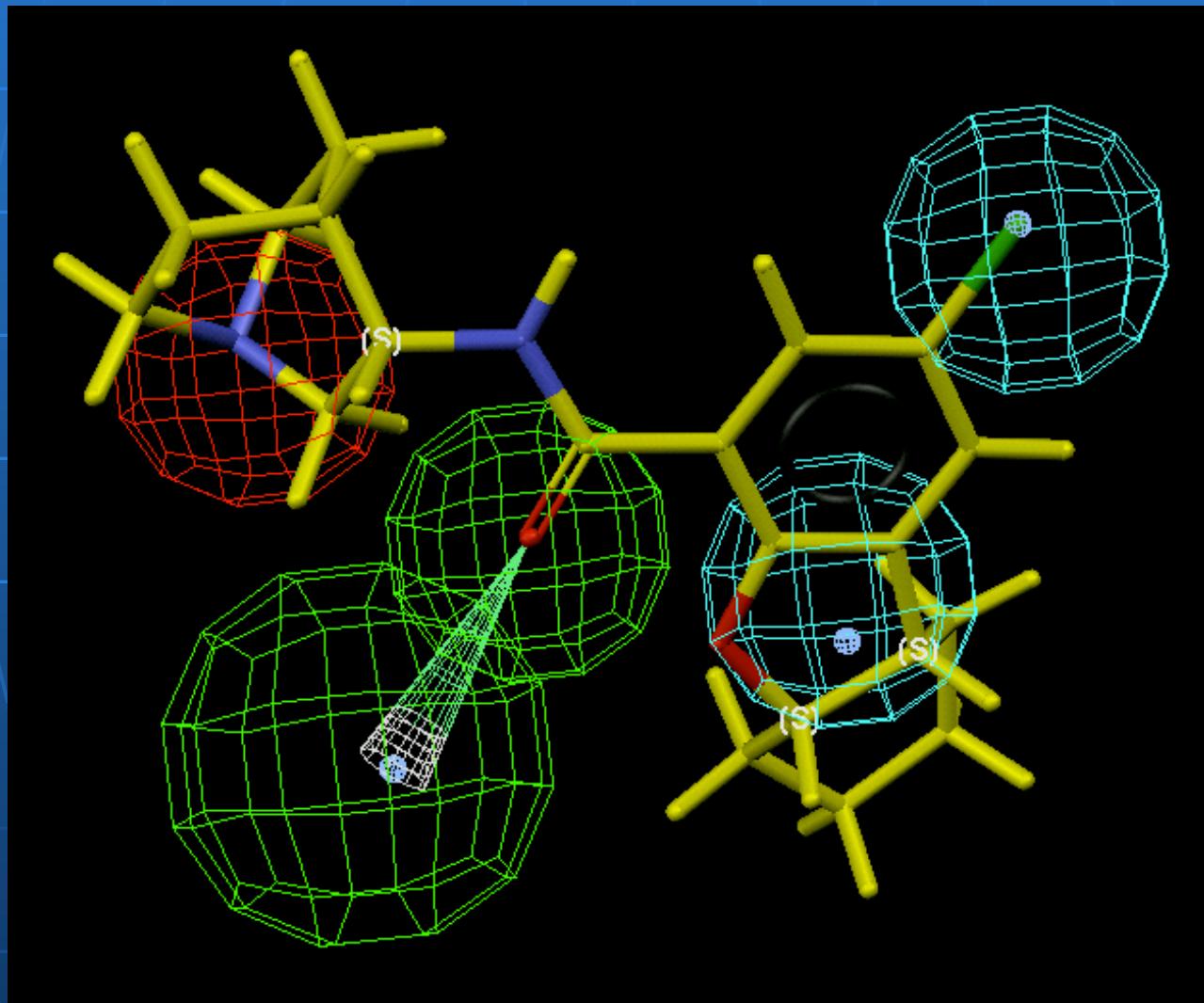
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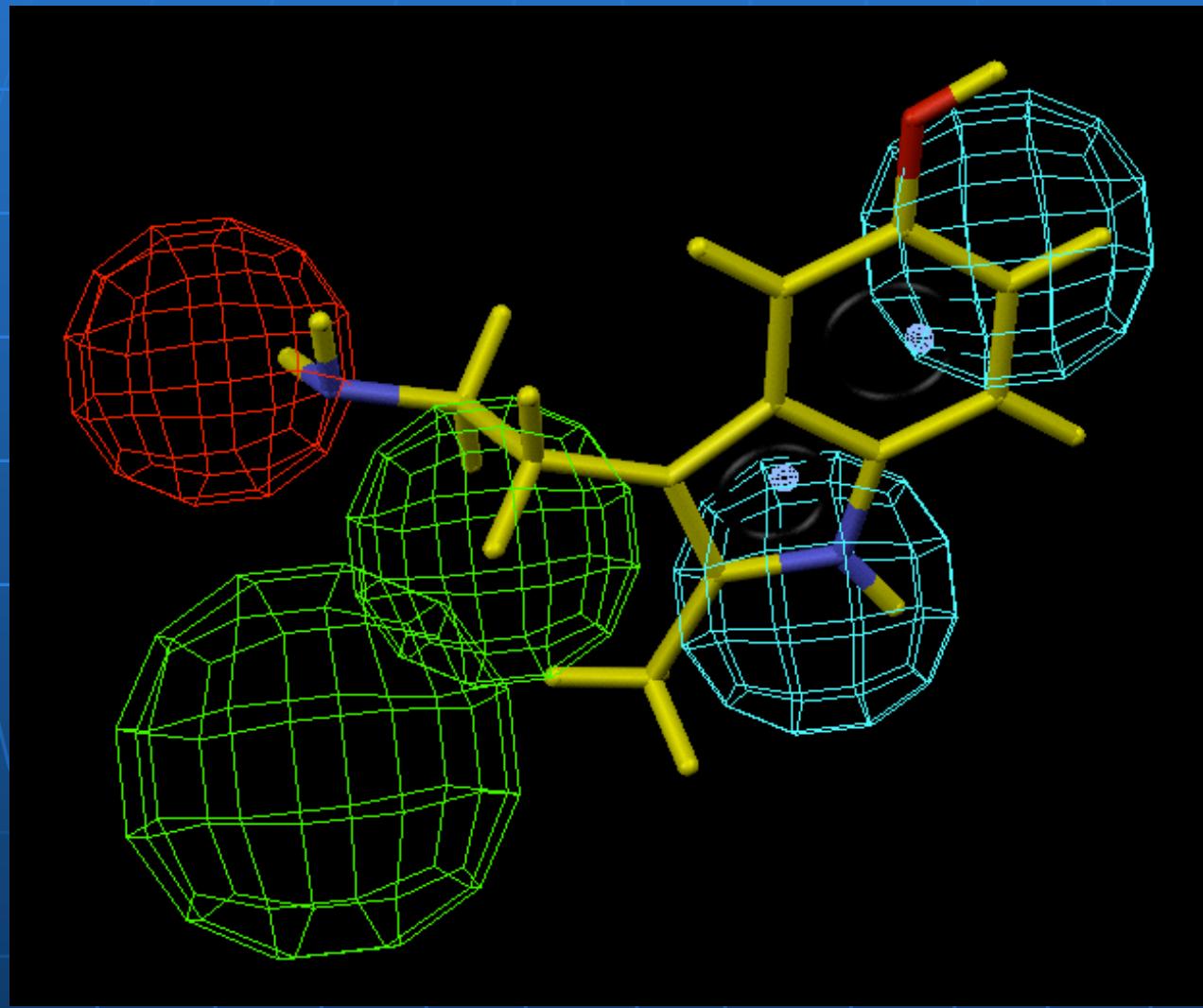
Constrained Conformational Search

- **Given a series of potent analogs**
 - Most rigid has fewest torsions → fewest conformers
 - Bind same site, common DMAP(s)
- **Scan, reject, evaluate DMAP**
 - Start with most rigid → keep all valid DMAPs
 - Next most rigid → keep only common DMAPs
 - Iterate until converge on DMAP, or run out of information in the analog series
- **Evaluate all analogs w.r.t. the most constrained DMAP (i. e., the final iteration)**
- **Also superconstrained case, same DMAPs**

Active (QSAR + Conformational Search)

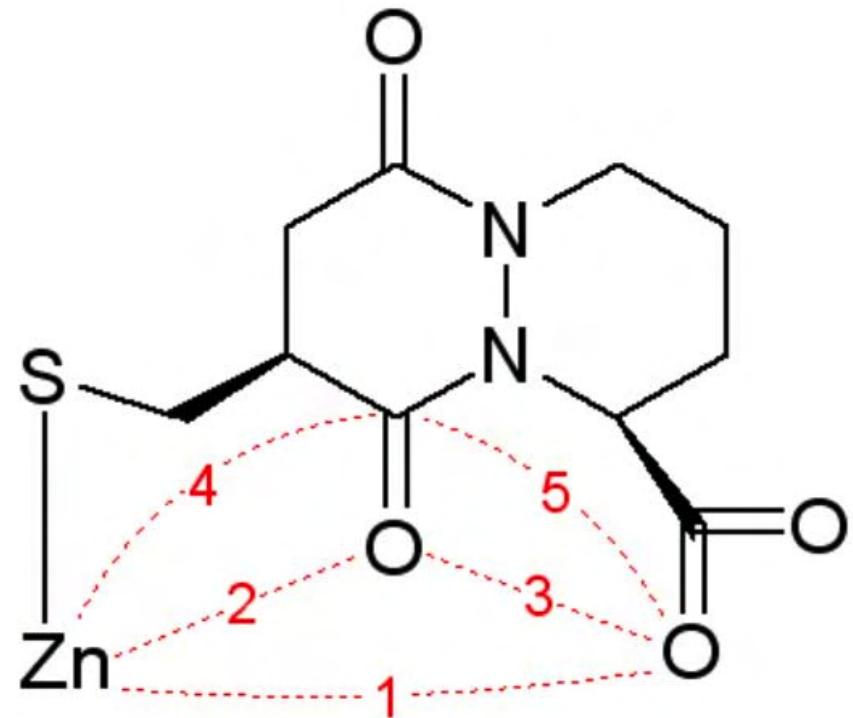


Inactive (QSAR + Conformational Search)



Convergence of DMAPs for ACE

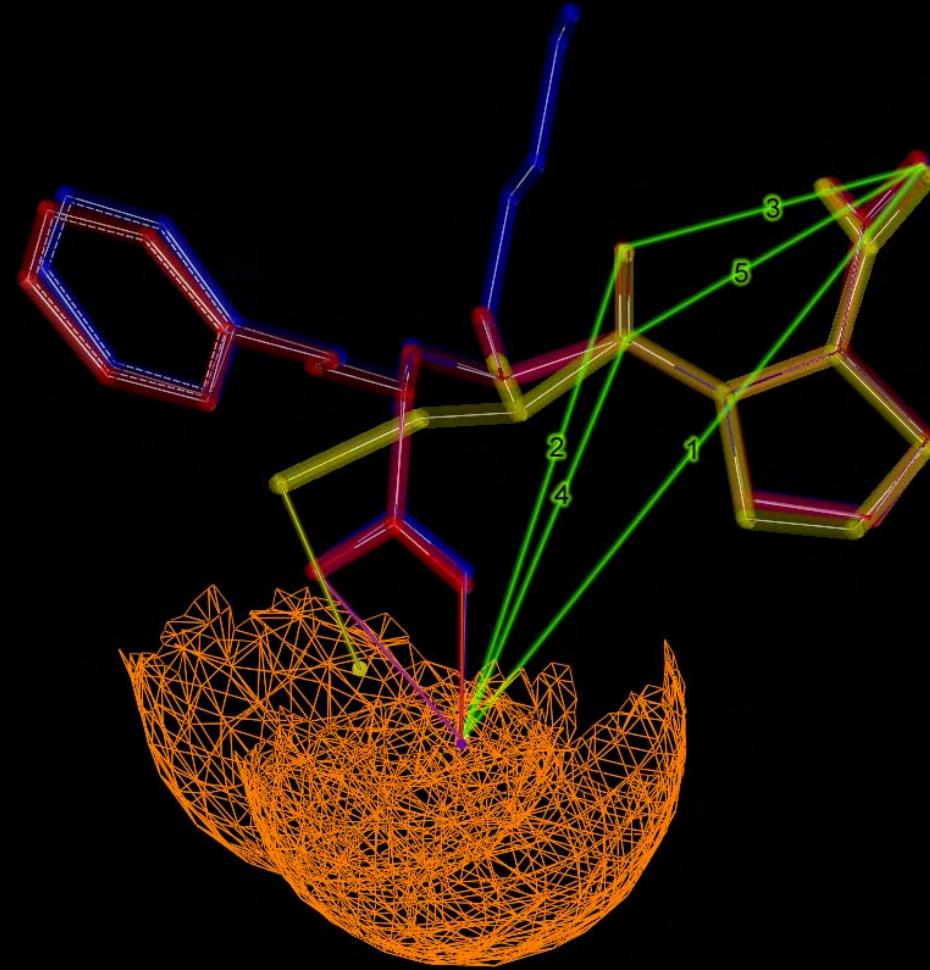
Comparison with 2004 crystal structure of captopril bound to ACE



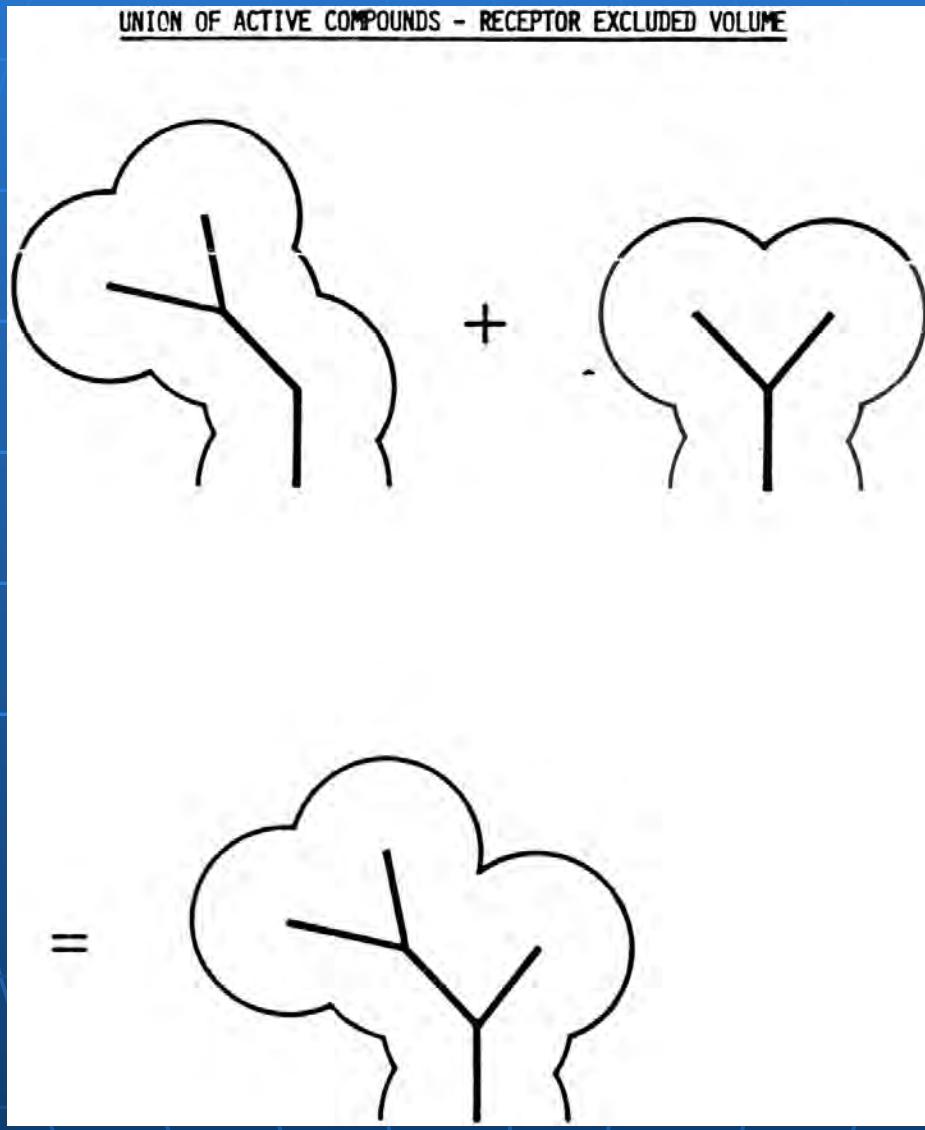
Predicted model	Crystal structures	Deviation
1: 7.188 - 7.812	8.485 - 8.673	~1.1
2: 4.812 - 5.312	5.718 - 5.981	~0.6
3: 3.562	3.532 - 3.628	~0.0
4: 4.812 - 5.062	4.876 - 5.181	~0.1
5: 3.938	3.989 - 4.047	~0.0

Comparison of Crystal Structures of 3 Bound ACE Inhibitors (2003, 2004)

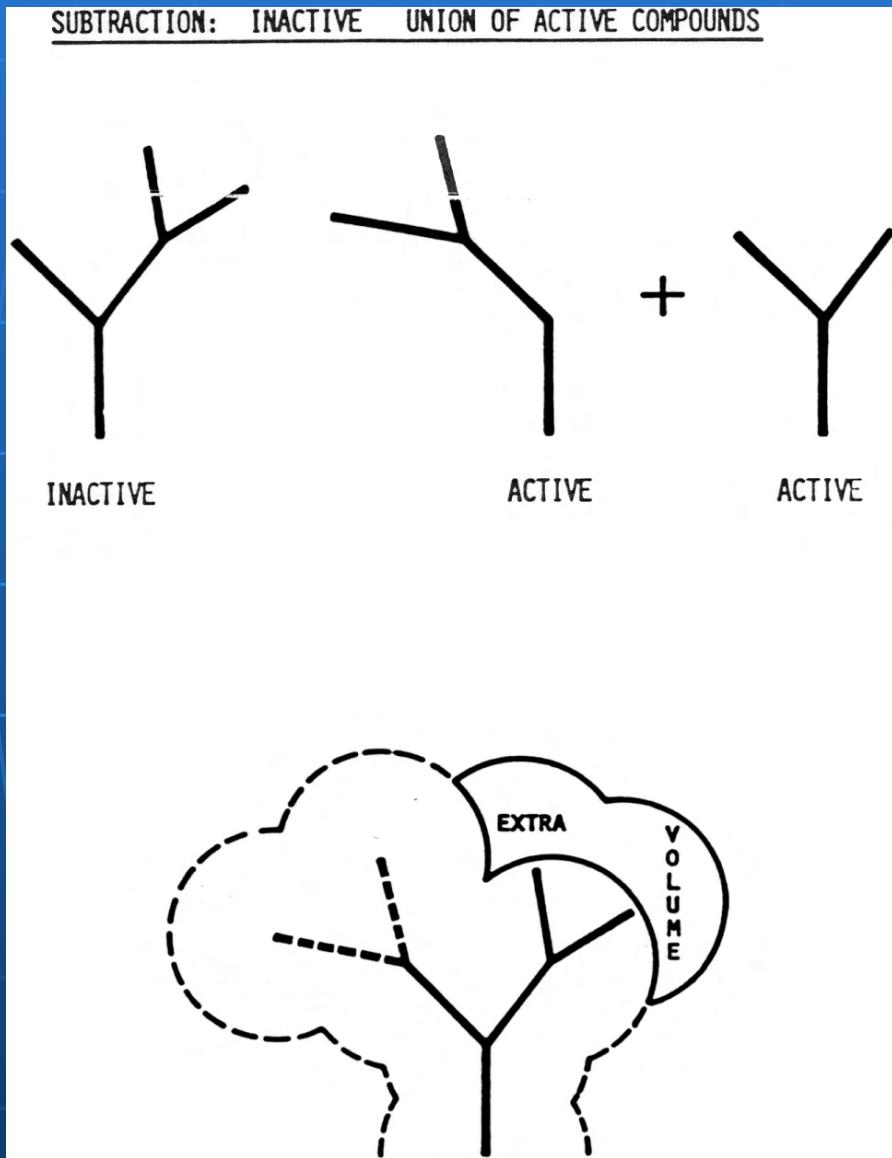
Overlap of 3 ACE Inhibitors (Captopril, Enalopril and Lysinopril) bound to ACE. Alignments are based on ACE structure. Note movement of Zinc (orange net) to accommodate SH vs COOH complexes



Analysis of Excluded Volume

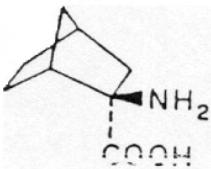


Analysis of Excluded Volume

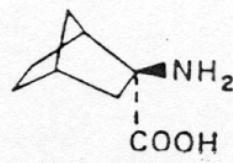


Example: Volume Mapping

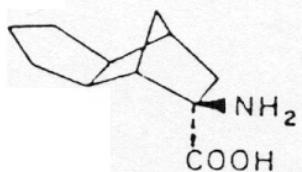
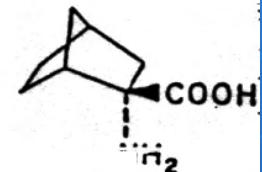
ACTIVE ANALOGS



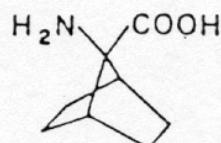
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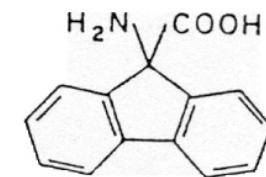
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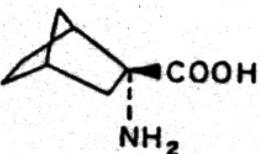
IV



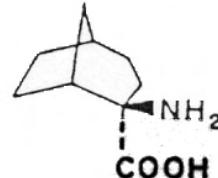
V



INACTIVE ANALOGS



VII



VIII

FIG. 1. Structures of active and inactive amino acid analogues used in volume mapping of the L-methionine binding site of methionine adenosyltransferase

Example: Volume Mapping

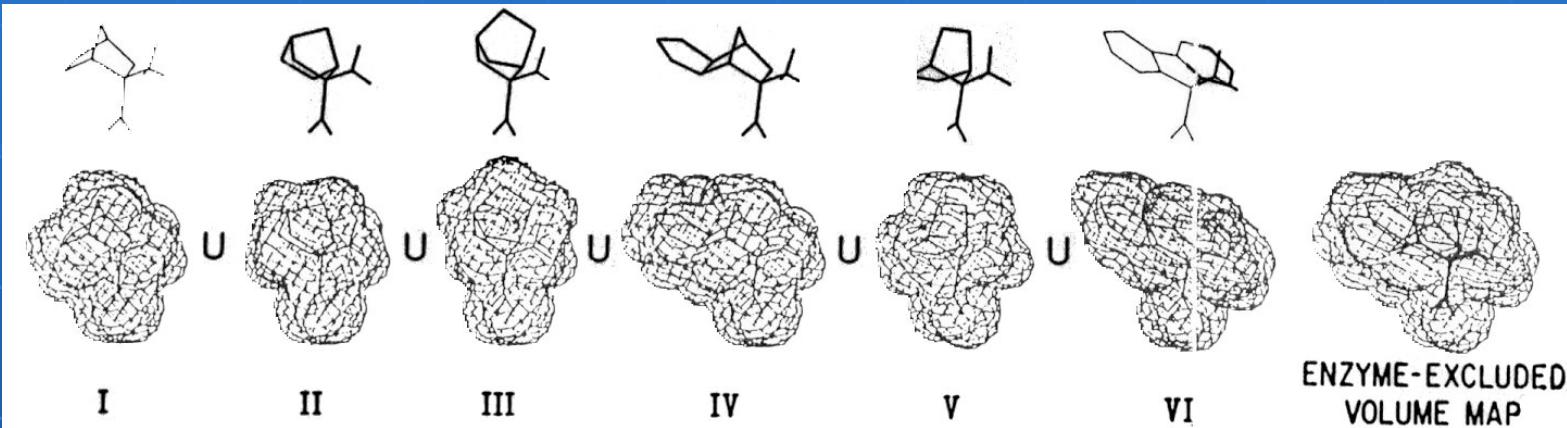


FIG. 2. Enzyme-excluded volume map

The structures of the six active analogues are shown on top; below each structure is the electron density map for that molecule. The union of these six individual electron density maps gives the enzyme-excluded volume map and defines that region of the methionine binding site available for binding by substrate, substrate analogues, or nonsubstrate analogues.

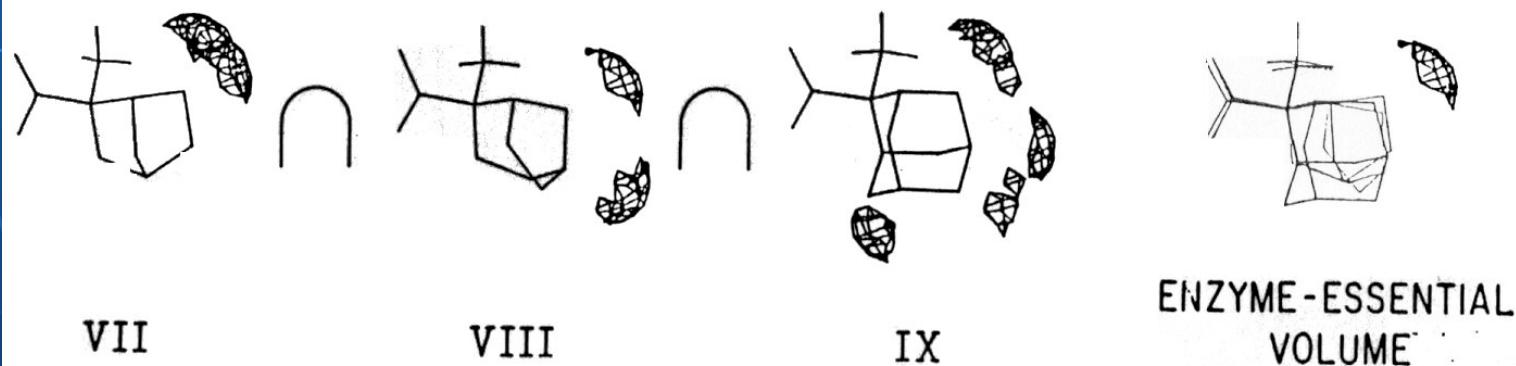
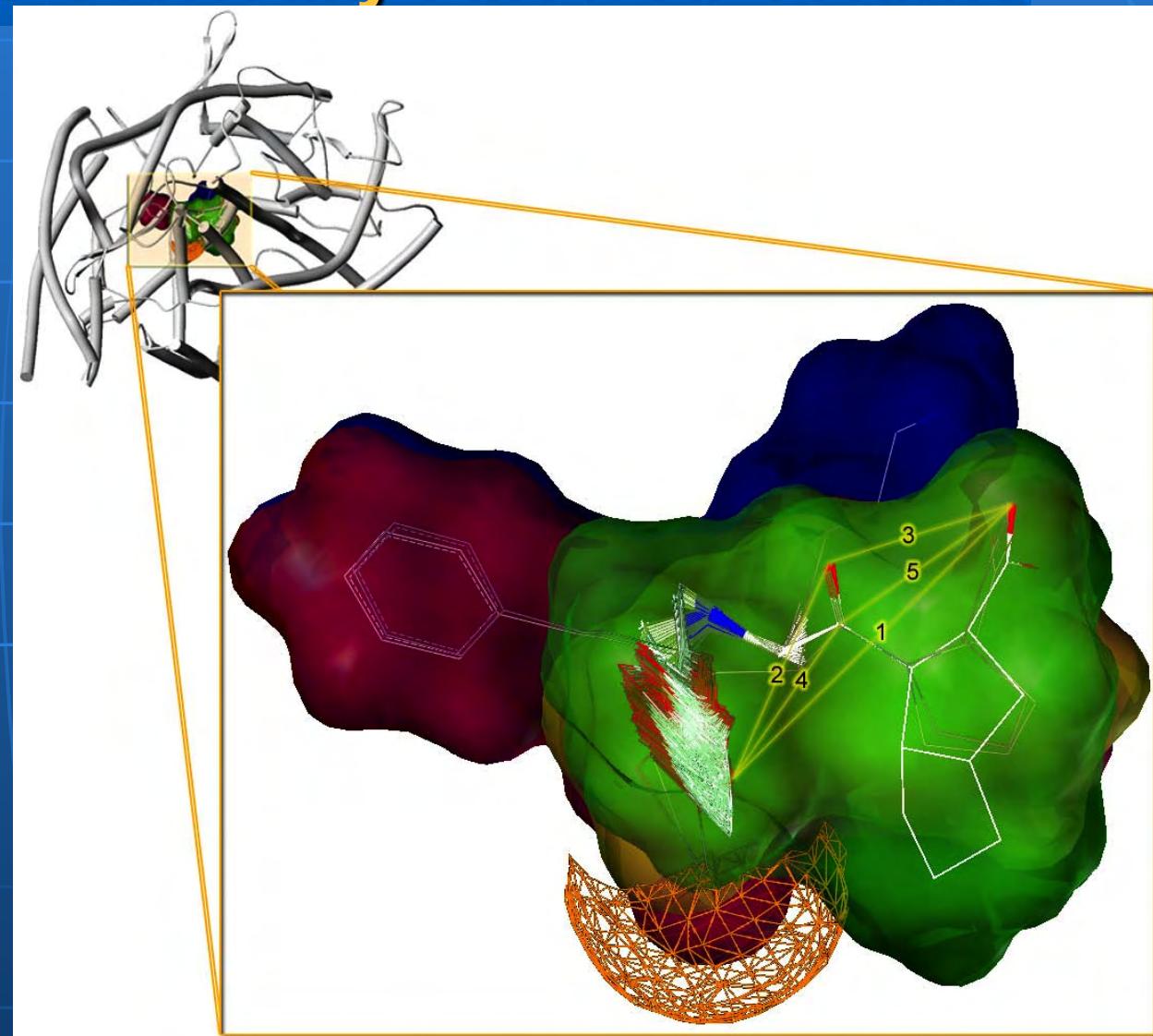


FIG. 3. Enzyme-essential volume map

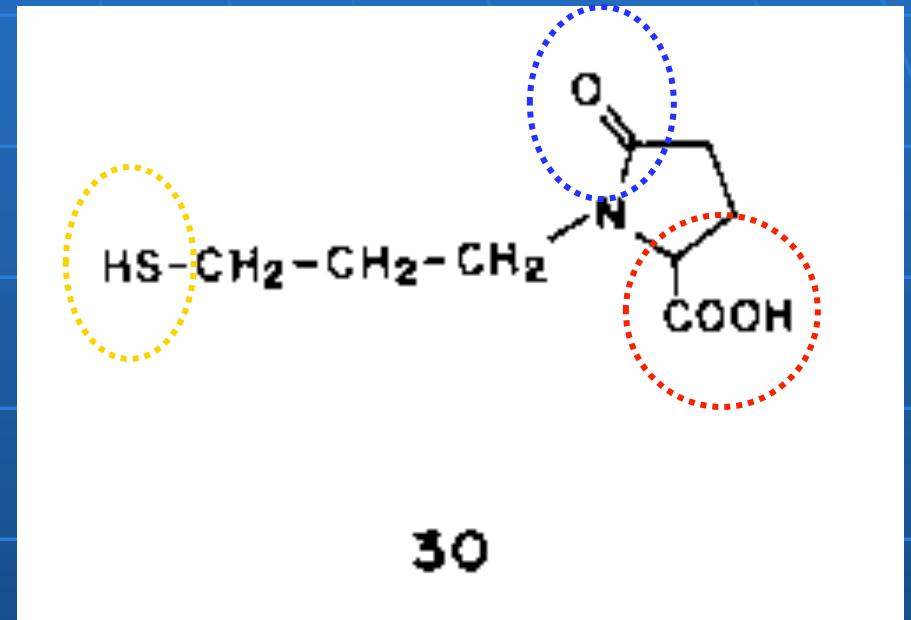
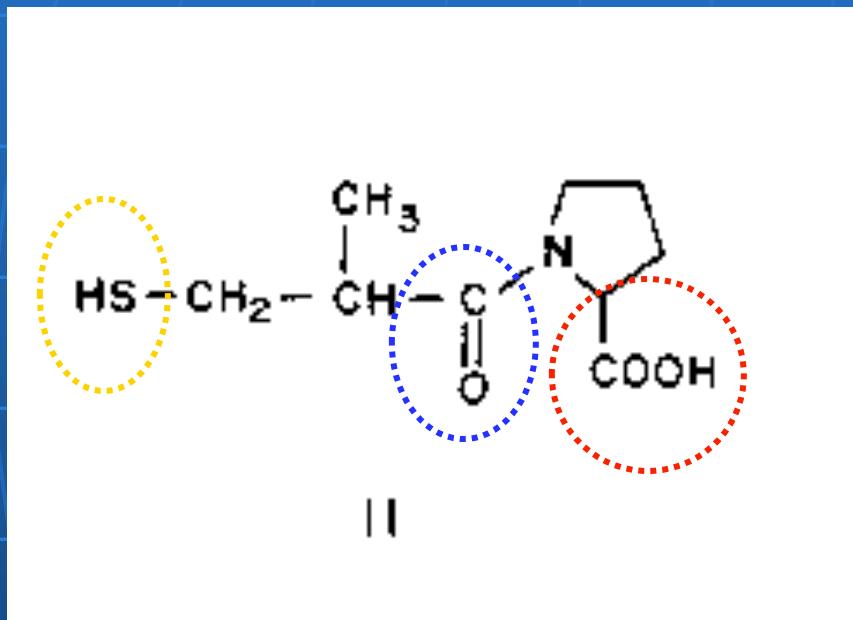
Each inactive molecule is shown with its unique volume segments that are not part of the enzyme-excluded volume map. Intersection of the unique volume segments of each inactive analogue gives one region of unique volume overlap for all three molecules which defines the enzyme-essential volume, i.e., a region occupied by the enzyme and therefore not available for occupancy by other molecules.

Use of Predicted Bound ACE Inhibitors to Map Enzyme Sterically Allowed Volume

One thousand two hundred and seventy five conformations of the inhibitor ramipril (Altace) predicted at the active site of ACE, compared with the experimental structures of lisinopril, enalapril and captopril. The Connolly molecular surfaces of lisinopril (blue), enalapril (red) and captopril (yellow) are shown to overlap significantly with the predicted ramipril structures (green). All three inhibitors bind the same identical active site on ACE; the active site anchor points coincide as predicted. The predicted ramipril conformations fit within the common volume of the experimentally determined inhibitors. The orange mesh represents an electron density contour encapsulating all zinc loci observed in the crystal structures. Only the constrained portion of ramipril is shown.

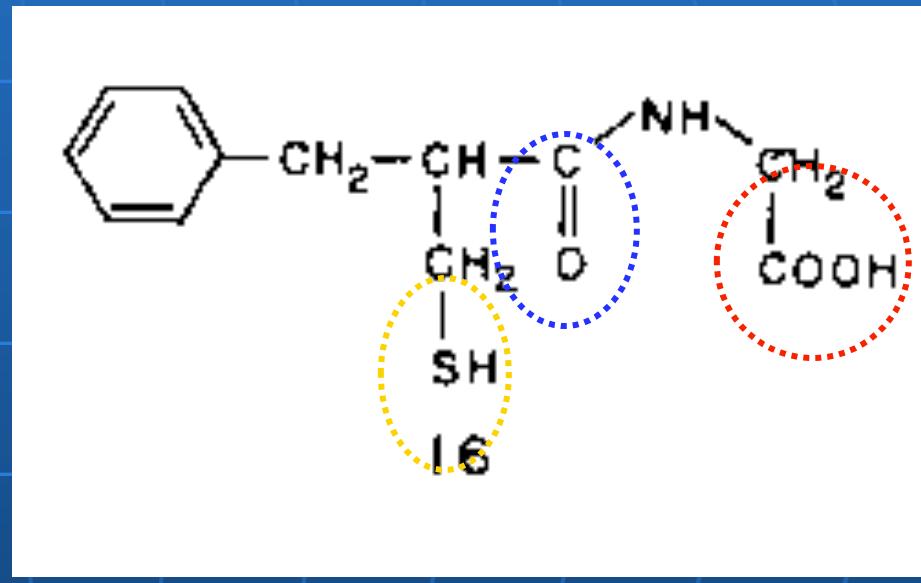
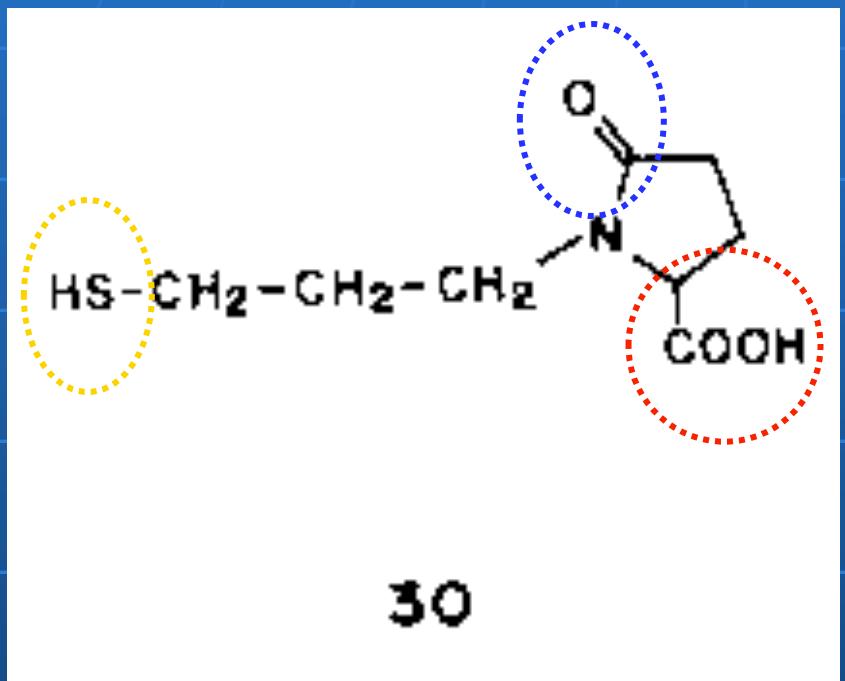


Quiz: which of these is active?



- How to judge whether or not a molecule might be active, based on only structural info?

Quiz: which of these is active?



- How to judge whether or not a molecule might be active, based on only structural info?
- Guess which one is active?

Lingo check

- **Conformational hyperspace**
- **Distance Map**
- **Sampling**
- **Resolution**
- **Active analog hypothesis**
- **QSAR**
- **Excluded Volume**



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Recap

- **What is a pharmacophore?**
- **Give an example strategy for sampling conformational hyperspace**
 - Pros/Cons to this strategy?
- **Quickly outline QSAR**
 - How derive constraints from physical interactions between ligand and receptor?
 - How could you use constraints to predict 3D conformations of a bound ligand?
 - Strategy for identifying a good (or bad) prediction of activity for a particular ligand?

Things to know, 90 minutes from now

- **What is a pharmacophore?**
 - Pattern recognition
 - Distance constraints
 - Degrees of freedom and conformational hyperspace
- **Quantitative structure-activity relationships (QSAR)**
- **Alignment rule for compounds is essential for 3D QSAR**
- **How to derive constraints from physical interactions between ligand and receptor?**
- **How to apply these constraints to predict active 3D conformations of bound ligands?**
- **How to identify a good (or bad) prediction**

Systematic Search Methodology

(Combinatorials Consume CPUs)

- 1. Prune combinatorial tree ASAP**
- 2. Divide and conquer (start at middle of graph)**
- 3. Use analytical approaches to determine solution space (where to look) rather than stochastic processes to find it**
- 4. Transform problem with constraints on solutions - start with most constrained example to provide tightest bounds on search**

Computational Complexity of Systematic Search

The conceptual simplicity of systematic search is in sharp contrast to the combinatorial complexity of its calculation. If A is the torsion angle increment and T is the number of rotatable bonds in the molecule, then the total number of possible conformers to be examined for steric conflict is $(360/A)^T$. If N is the number of atoms in a molecule, $N(N - 1)/2$ pairwise van der Waals evaluations must be done for each conformation. Consequently, the number of pairwise van der Waals evaluations, V , required for a molecule during the course of a systematic search is given by:

$$V = \left(\frac{360}{A}\right)^T \times \frac{N(N - 1)}{2}$$

Table 1

Relative computational complexity of systematic search as a function of the number of torsions and the angle increment. These values were determined using the expression for V (Eq. (1)) and simple hydrocarbons — 5 torsions corresponds to hexane, 10 torsions corresponds to undecane, etc.

Number of torsions	5	10	20	40
Angle increment				
30	1	9.1×10^5	2.1×10^{17}	3.2×10^{39}
15	3.2×10^1	9.1×10^8	2.2×10^{23}	3.5×10^{51}
8	7.4×10^2	5.0×10^{11}	6.5×10^{29}	2.9×10^{62}
4	2.4×10^4	5.0×10^{14}	6.81×10^{35}	3.2×10^{72}
2	7.6×10^5	5.3×10^{17}	7.1×10^{40}	3.5×10^{86}

Combinatorial Tree Truncation

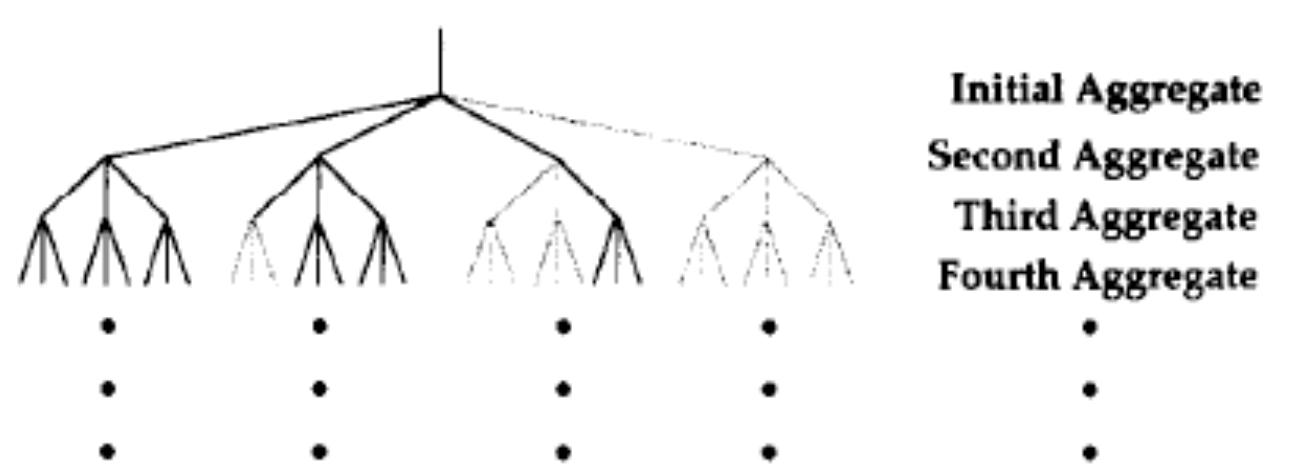


Fig. 1. The tree structure of systematic search. At each branch point, the possible addition of a new aggregate to the existing partial conformation is evaluated for steric contacts. Gray lines represent “pruning” of the search tree by eliminating from further consideration those branches in which the addition of an aggregate is not sterically allowed at any torsion setting. The process continues until the addition of all aggregates along every branch has been considered.

Analytical Truncation

Distance constraint equations describe the variable distance between any two atoms as a function of a single torsion angle. The square of the interatomic distance between a_j and a_i is given by:

$$d_{ij}^2(\omega) = d_1 + d_2 \cos \omega + d_3 \sin \omega \quad (2)$$

where the coefficients are defined as follows:

$$d_1 = |s|^2 + |v|^2 - 2(s \cdot v_1), d_2 = -2(s \cdot v_2), \text{ and } d_3 = -2(s \cdot v_3).$$

v_1 , v_2 , and v_3 are the three orthogonal components of the vector v in Fig. 2 ($v_3 = v \times u$, $v_2 = u \times v_3$, and $v_1 = v - v_2$).

By a substitution of variables ($x = \tan \frac{\omega}{2}$), Eq. (2) simplifies to

$$d_{ij}^2(x) = \frac{(ax^2 + bx + c)}{(1+x^2)} \text{ where } a = d_1 - d_2, b = 2d_3, \text{ and } c = d_1 + d_2. \quad (3)$$

The values of x which minimize or maximize $d_{ij}^2(x)$ are given by

$$x = \pm \frac{\sqrt{d_2^2 + d_3^2 - d_2}}{d_3}$$

These values of x may be substituted into Eq. (3) to find the minimum and maximum distances between atoms i and j as a function of the torsion variable.

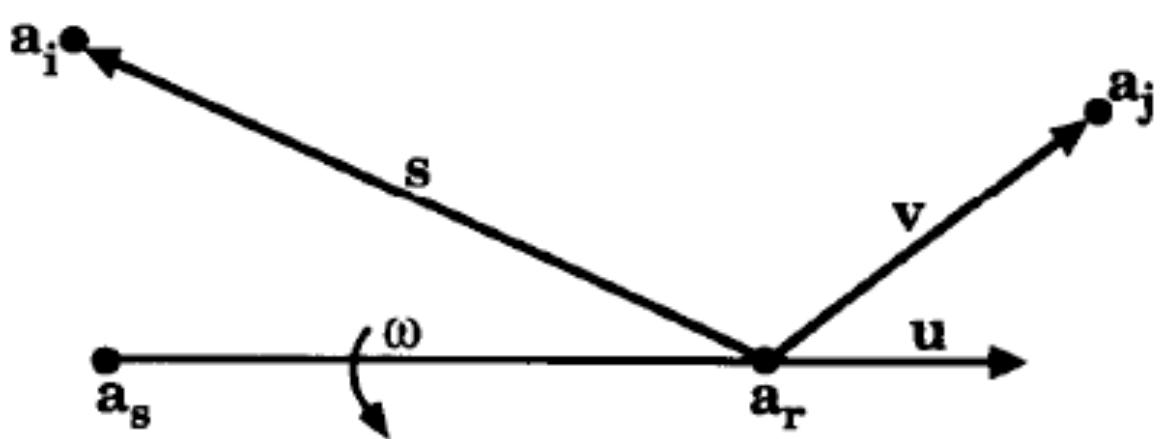


Fig. 2. Geometric definitions in the evaluation of steric contacts using the distance constraint equations during the addition of an aggregate to a sterically allowed partial conformation: a_i , a_s and a_r belong to the sterically allowed partial conformation and are rigid with respect to each other; a_s , a_r and a_j belong to the aggregate which is being added; a_s and a_r are atoms at the end of the torsion (ω) under consideration; s and v are vectors as shown; and u is a unit vector along the axis of rotation.

Analytical Truncation #2

If c_{ij} is the sum of the van der Waals radii for two non-bonded atoms i and j, the differential distance function, $\delta_{ij}(\omega) = d_{ij}^2(\omega) - c_{ij}^2$ is evaluated to determine whether or not the two atoms are in contact. When the differential distance function is converted to quadratic form, the resulting discriminant, $D = b^2 - 4(a - c_{ij}^2)(c - c_{ij}^2)$, can be used as a filter to determine the fate of each new aggregate. If $D > 0$, then δ_{ij} has real roots, indicating that a given atom pair will be in contact for some torsional ranges. The endpoints of these ranges can be calculated for that pair using the expressions above and removed from consideration for other atom pairs. If $D \leq 0$, δ_{ij} has complex or real double degenerate roots. For $c - c_{ij}^2 \geq 0$, $\delta_{ij}(\omega)$ is positive for all values of ω and δ_{ij} has only

imaginary roots, implying that a_i and a_j never come in contact for any value of the torsional rotation. This pair of atoms can be removed from consideration in the addition of the new aggregate. For $c - c_{ij}^2 < 0$, $\delta_{ij}(\omega)$ is negative for all values of ω and there is no sterically allowed way to add the aggregate. In this case, the new partial conformation has to be discarded and the search branch truncated.

The distance constraint equations minimize the number of pairwise distances that must be evaluated in a systematic search. They prune the search tree by analytically determining torsion angle ranges that may contain sterically allowed conformers. The intersection of the allowed angle ranges for every atom pair spanning a torsion defines the torsion ranges in which conformers must actually be built and evaluated to determine if they are sterically valid. Based on these angle ranges, trigonometric tables are constructed to guide the rotation matrices that govern the construction of conformations.

Analytical Truncation #3

Ranges of ω_0 and ω_1 which are consistent with the maximum and minimum allowed distance between A_0 and A_1 , have already been determined using two successive applications of the distance equations. In order to determine ranges of ω_0 which are consistent with the constraint between A_0 and A_2 , A_2 is projected onto the ω_2 axis (P_2), and this projection is in turn projected onto the ω_1 axis (P_3). The $A_0 - A_2$ distance constraint is expanded by $d_2 + d_3$ and used in the distance equations to find allowed torsion ranges for ω_0 . In principle this approximation could be extended over a greater number of rotatable bonds, but in practice the adjustment to the distance constraint reduces its information content. With the exception of cyclic structures, look-aheads extending beyond three rotatable bonds down the search tree do not significantly refine the allowed ranges for the initial rotatable bond. Our experience is that the look-ahead feature contributes speed enhancements of 20- to 100-fold. Look-aheads are used at many points in the search process especially for cyclic structures, in some cases to filter entries from the existing trigonometric table of allowed angles.

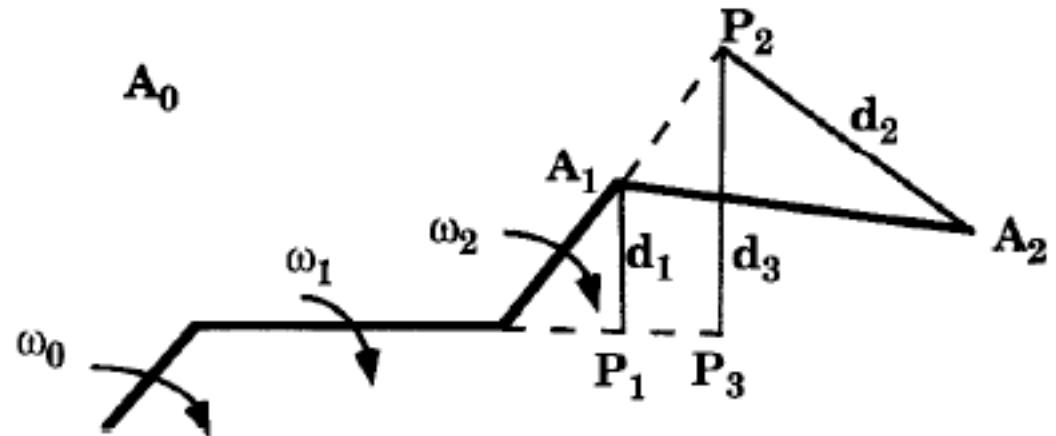


Fig. 3. Geometric parameters used in the look-ahead capability of systematic search. A_0 is an atom in the partial conformation preceding ω_0 ; A_1 and A_2 are atoms in the aggregates following ω_1 and ω_2 , respectively; P_1 is the projection of A_1 onto the ω_1 rotation axis; P_2 is the projection of A_2 onto the ω_2 rotation axis; d_1 is the radius of the A_1 rotation circle around ω_1 ; d_2 is the radius of the A_2 rotation circle around ω_2 ; P_3 is the projection of P_2 onto the ω_1 rotation axis; and d_3 is the radius of the P_2 rotation circle around ω_1 .

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