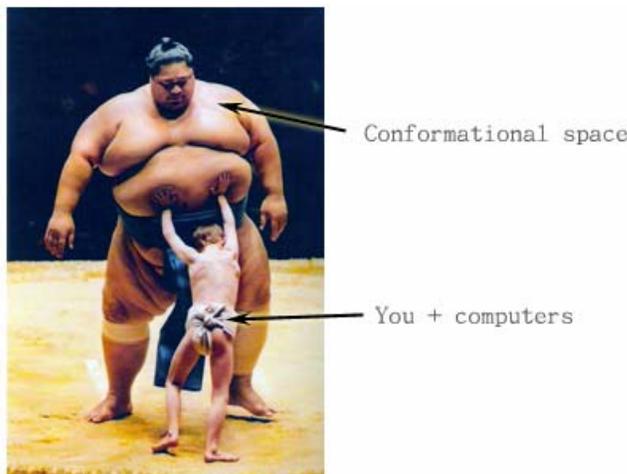


Conformational search and QSAR (quantitative structure-activity relationships)



Purpose:

In this lab, you will use SYBYL to build, visualize, and explore conformations of drugs. The goal is to extract information inherent in the conformations of a series of drugs, to construct a hypothesis for how ACE inhibitors bind to the receptor (ACE). Work alone or in groups of two.

This lab assumes you are familiar with Mac OS. In addition, the molecular modeling package SYBYL is introduced—it will be used for several upcoming labs as well. SYBYL is a powerful environment, and you will see only a fraction of its functionality in the labs...feel free to peruse other tutorials on your own (outside of lab class today) if you want to learn more!

Lab outline:

- 0) Listen to 10 minute intro on ACE/conformational search/QSAR; ask questions if unclear!
- 1) Set up your MacOS environment for SYBYL
- 2) Follow along (as a class) with SYBYL basics tutorial
- 3) Do the Sketch tutorial
- 4) Do the Conformational Search & Distance map tutorials
- 5) Answer the questions at the end

This lab should take 2-3 hours total, if it is taking you much longer, ask questions!

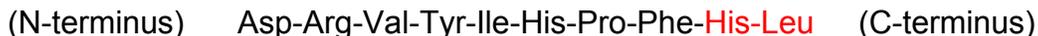
0.1 Biological background:

ACE (angiotensin converting enzyme) is a protein in your body that participates in the angiotensin-renin-aldosterone system for controlling blood volume and pressure. Like many proteins, it has several functions in the context of the system, and those interested in systems biology would probably find this system worth studying, or at least surveying.

Today we will focus on the two major functions of ACE: i) conversion of the decapeptide angiotensin I to the octapeptide angiotensin II by cleavage of a carboxy terminal His-Leu dipeptide; ii) inactivation of bradykinin, a nonapeptide vasodilator.

ACE (EC 3.4.15.1) is a exopeptidase that relies on a catalytic zinc and active site waters to hydrolyze peptide bonds near the C-terminus of a bound peptide. Increased amounts of angiotensin II shifts the equilibrium of angiotensin-renin-aldosterone system towards higher systemic blood pressure. Bradykinin is a vasodilator, so inactivation of bradykinin prevents vasodilation and suppresses that pressure-lowering effect. Therefore, mechanistic targets for lowering blood pressure include the inhibition of angiotensin II receptors and ACE. ACE inhibitors are perhaps the first example of rational drug design¹ and they have proven more effective clinically than angiotensin receptor blockers. ACE inhibitors are a major molecular therapy against hypertension, and have been a \$billion/year industry for 10+ years. Popularly prescribed ACE inhibitors include: captopril (now generic), enalapril (Vasotec[®]; Merck), lisinopril (Zestril[®]; Astra-Zeneca and Prinivil[®]; Merck), ramipril (Altace[®]; King/Aventis), quinapril (Accupril[®]; Pfizer), trandolapril (Mavik[®]; Abbott) and others.

The sequence of the native ACE substrate, angiotensin I, is shown below. Cleavage of the red His-Leu C-terminus yields angiotensin II.

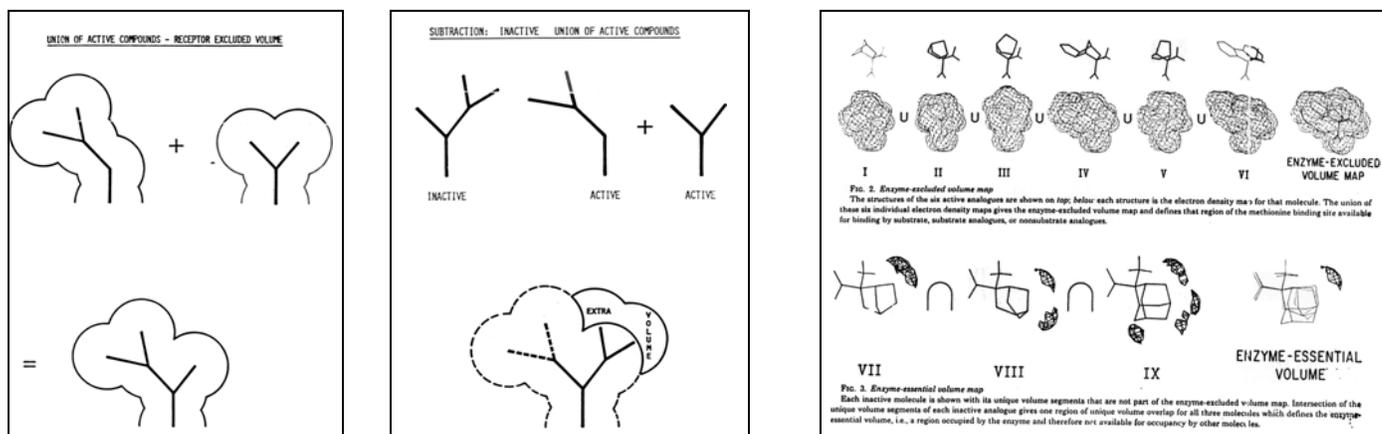


There are several relevant known interactions between the ACE active site and bound inhibitors. These features were derived from structure-activity studies, and seem to be necessary for binding. Thus, the three points below constitute a pharmacophore:

- i) terminal carboxyl group (COOH) for ionic interaction with a positively charged residue on ACE
- ii) carbonyl (C=O) to participate in assumed hydrogen bonding with ACE
- iii) a functional group to coordinate a Zn at the active site

0.2 Conformational search background

If a series of inhibitors all bind to the same (rigid) active site, they must share some geometric features. Ligand-based hypotheses like systematic search and QSAR are an extension of this idea, to address the question: Given that molecules are flexible and can adopt many conformations, which particular conformations are sterically allowed? And is there a subset of the allowed conformations that is common to all ligands?



¹ Though the process for studying angiotensin analogs and designing ACE inhibitors was pioneering, much of the work was done by analogy to thermolysin and carboxypeptidase A, which was hypothesized to have a similar active site. After the ACE structure was published in 2003, this was not shown to be the case, but nevertheless, the resulting ACE inhibitors are effective.

A simple systematic search is a trial-and-error exhaustive sampling of all sterically allowed conformations. "Systematic" implies that the search space is covered in a regular and predictable way. There is some subset of these conformations that bind the active site and are "active." A constrained systematic search adds a set of constraints that must be in common to all active binders. This intersecting set of conformations represents possible active conformations. SYBYL has nice constrained search and analysis tools, so it is our weapon today.

Conformational space is HUGE. The number of conformations in a molecule is described by:

$$N = \left(\frac{360}{\delta} \right)^{nbonds}$$

where N = # conformations
 δ = rotation increment in degrees
nbonds = # of rotatable bonds (degrees of freedom)

For a small, 40-residue protein with all alanine sidechains (so only backbone phi/psi degrees of freedom matter), at 10° increments, this gives 3.2×10^{124} conformations. For reference, the age of the visible universe is probably about 10^{18} seconds. This is why naïve brute-force folding of macromolecules will probably never be possible.

How about a small molecule with 6 rotatable bonds? $N = 2.18 \times 10^9$. This is near the limit of what a single Pentium/Core Duo desktop machine with 1 gigabyte of RAM can search unconstrained and analyze using SYBYL. That's a good start, but many interesting molecules have more than 6 rotatable bonds, so it is necessary to apply constraints to attack this problem.

1.1 Setup your Mac OS environment for SYBYL

1.1.1 Correct mouse behavior

SYBYL relies on 3 button mouse operation, but Apple sees fit to give you only a crippled 2 button mouse. Left-click should be fine as default. To modify the right-click to work with SYBYL:

System Preferences > Keyboard & Mouse > Re-map right button to "Button 2" or "secondary"

Ideally, you could re-map the middle mouse button to "Button 3" in the same way, but it appears this feature is broken in MacOS. Buttons are handy.

1.1.2 Copy files

You may need to use the GUI or command line to copy files for the various SYBYL tutorials. The terminal application is located at: Mac HD > Applications > Utilities > Terminal. It is handy to put the Terminal shortcut on your toolbar at the bottom (drag & drop). You don't need to copy any other files for the conformational search tutorial.

1.1.3 Load Tripos Bookshelf tutorials

Tripos has created a very nice set of tutorials for the various SYBYL functionalities. To load the Tripos Bookshelf, open a web browser (Safari) and type in the address bar:

`file:///usr/local/sybyl8.1/TriposBookshelf/index.html`

It is handy to make a bookmark for Tripos Bookshelf.

1.1.4 Launch SYBYL

SYBYL needs certain environment variables to work correctly. To set them up automagically, open a new terminal window and type:

```
trigo -shell sybyl8.1
```

This spawns a new shell (in the same window) where SYBYL can run. To launch SYBYL, type:

```
sybyl
```

2.1 SYBYL basics

Follow along with the class for the basics introduction. If you want to do this again later, or on your own, you can use the Tripos Bookshelf tutorial for "SYBYL Basics" on the main page, in the "Sybyl Core Technologies" box, second down on the left. The Introduction and Small Molecule Sketching tutorials are the most useful.

3.1 Do the Small Molecule Sketching tutorial on your own, here in class

"Sybyl Core Technologies" > SYBYL Basics > Small Molecule Sketching

4.1 Do the Conformational Search and Distance Map tutorials on your own or in pairs

"Cheminformatics" box (bottom right) > Conformational Analysis > Systematic Search
Distance Map

No need to do the Conformational Comparison tutorial!

5.1 Questions

- 1a. Sketch the molecule benzamidine in SYBYL.
- 1b. Sketch L-alanine in SYBYL. Is the chirality correct?
- 1c. Invert your L-alanine to D-alanine. Verify chirality.

2. Generate Connolly molecular surface visualizations for benzamidine. Map some useful property onto that molecular surface (ie, electrostatic potential, curvature, etc)

3. Consider the concepts of a Ramachandran map for amino acids, versus systematic search in small molecules. Similarities? Differences?

4. Given some molecule that satisfied the three essential structural ACE inhibitor features (i,ii,iii on p.2), how would you go about testing the hypothesis that it can inhibit ACE?

4. Bonus! Imagine you hadn't known the three structural requirements for ACE, but you did have a large series of active inhibitors. How could you deduce the pharmacophoric points using a constrained search?

You don't need to turn in anything for this lab, but make sure you understand the concepts. Later labs will depend on you being able to operate a constrained systematic search and test pharmacophore hypotheses!