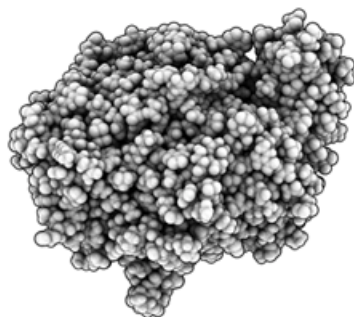# Identifying active molecules from a series of decoys

Purpose:

This is a practical lab, no more tutorials! Your task is to identify three active ACE inhibitors from a set of ten decoys. All the skills/tools/knowledge necessary to do so have been introduced in previous labs. Treat this lab as a "real world" exercise in molecular modeling!

Goals for this lab:
0) Listen to 10 minute intro, ask questions if unclear
1) Establish an attack plan for yourself, answer questions 1 & 2
2) Use computational tools to execute your plan
3) Identify 3 active compounds
4) Answer remaining questions
5) Submit your answers to Tim or Dan in person or by email

(Due date: 12-Dec-2008 at noon)

The decoy set and some supplementary materials are available on the following pages. There is no single way to attack this challenge! It may be possible to "game" the lab by looking through lists of active drugs to identify ACE inhibitors…but this approach is not instructive and will not help you answer the questions. Collaboration is encouraged as long as you do your own work and submit your own answers!

Previous labs have focused on some central concepts in molecular modeling:
  Minimization (TINKER, SYBYL)
  Molecular Dynamics (TINKER, SYBYL)
  Small Molecule Docking (Autodock)
  Electrostatics & complementarity (APBS, TINKER, SYBYL)
  Homology Modeling (Modeller, SYBYL)
  Brownian Dynamics (SDA)
  QSAR & Conformational Search (SYBYL)
  Ligand Sketching & Optimization (SYBYL, RACHEL, TINKER)
  De novo design (SYBYL, RACHEL)

You should be comfortable using tools to manipulate molecules *in silico*, and to test hypotheses. Like a skilled mechanic can work on many autos, comfortably using the right tool to diagnose problems…the molecular modeler should be able to work on many biological systems, applying the right tool to discriminate hypotheses.

----------------------------------- (answer questions below immediately after introduction) -------------------------------

Question #1: Establish a hypothesis that explains, in atomic detail, what makes an ACE inhibitor "active"

Question #2: Outline an "attack plan" that will allow you to test your hypothesis using computational molecular modeling tools. (Hint: good *in silico* experiments are like good "wet lab" experiments; how will you test your hypothesis?)

----------------------------------- (do computational analysis, then answer questions below) -------------------------------

Question #3: Systematic search is a method for exploring the conformations of a flexible molecule that do not self-penetrate. Applying distance constraints can reveal atomic features that are shared amongst molecules in a series. How can distance maps be used to test a structure-activity hypothesis and discriminate between active and inactive conformations?

Question #4: Docking is a method for exploring how a ligand can fit into a receptor pocket. What additional constraints does docking incorporate, versus systematic search?

Question #5: What is the fundamental difference between systematic conformational search and the conformation search engines used in all docking algorithms today? (Hint: systematic search is prone to combinatorial explosion with ligands having >6 rotatable bonds, where docking is routinely done for much larger ligands!)

Question #6: Can you identify "by eye" any of the 10 decoys that are clearly not active? Did you try to do so before running your *in silico* tools? Why (or why not) is this good to do?

Question #7: Imagine you are a molecular modeler extraordinaire! One morning, your molecular biologist colleague (Juliette) tracks you down, having read in "The Scientist" that red wine contains a chemical to reverse aging. Juliette is sure that there is a molecular explanation for the anti-aging effects observed in mice treated with resveratrol (a compound extracted from grape skins and found in red wine). She even has a favorite receptor target for the resveratrol ligand--a family of proteins called sirtuins. Juliette wants you to do some computational analysis to help her design good, informative "wet lab" experiments to test whether or not resveratrol is active against sirtuins. What information do you need (from Juliette or elsewhere) before you begin your analyses? What type of analysis is useful here?

(10 points per question)

Supplementary materials:

Ligand-based hypothesis:
You have done a lab on QSAR and conformational searching, which holds that a series of active molecules must all share some geometric characteristics if they bind at the same (rigid) site. ACE has a rigid enzymatic active site. Ligand-based hypotheses like systematic search is 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 which might be active?

Receptor-based hypothesis:
You have also done a docking lab, using Autodock. Docking aims to find favorable configurations of ligands, packed into a receptor's active site. Most people computational chemists think about docking in two separable problems: conformational search and scoring.

Biological background on ACE:
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.

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 blocking angiotensin II receptors and inhibition of ACE enzymatic action. ACE inhibitors are perhaps the first example of rational drug design and they have proven more effective clinically than angiotensin receptor blockers. ACE inhibitors are a major molecular therapy against hypertension, and are a $billion/year industry. 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. Angiotensin II does not include the red His-Leu C-terminus.

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

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.

 a) a terminal carboxyl group to satisfy ionic interactions with a positively charged residue on ACE
 b) a carbonyl to participate in assumed hydrogen bonding with ACE
 c) a functional group to coordinate a Zn at the active site

The crystal structure of ACE, with no ligand bound, and also in complex with captopril, enalapril and lisinopril was solved in 2003.[1] These structures are available in the Protein DataBank (http://www.rcsb.org/pdb):

1O8A.mol2: ACE, no ligand bound
1O86.mol2: ACE + lisinopril
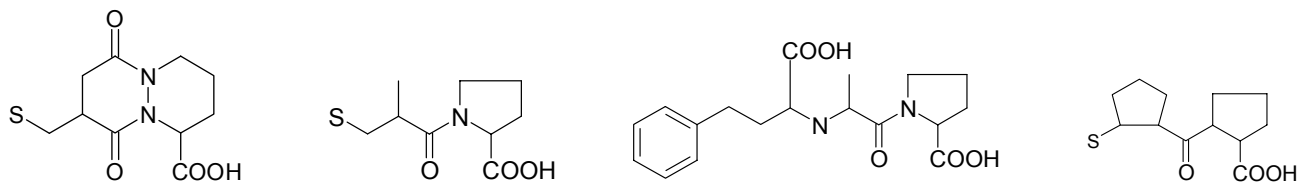1UZE.mol2: ACE + enalapril
1UZF.mol2: ACE + captopril

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[1] Natesh et al. (2003) Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature. 421:551-554.

Active and Decoy molecules:

Four known active inhibitors of ACE:

Known zinc-coordinating groups: carboxylate (COO-), hydroxamic acid (R-CO-NH-OH), phosphate, sulfhydryl (SH). All chiral centers are in peptide-mimicking S configuration unless noted, as (R).

And your 10 molecule set, of which three are active ACE inhibitors and seven are decoys.