



# IRON

## Software for easy computation of protein-ligand binding free energies

**I**ron is software for computing protein-ligand interaction free energies, easier to use than docking programs while yielding accuracies comparable to free energy perturbation (FEP) calculations.

Iron computes both the enthalpy and the elusive entropy of interaction, providing a better estimate of ligand-protein binding than enthalpy alone. Entropy terms in Iron account for motion of the protein, positioning of explicit water molecules, and conformational flexibility in the ligand, as well as unbiased sampling of ligand binding poses.

The Iron software uses the standard and validated AMBER force field for computations. However, unlike other free-energy computation programs, the program requires only an SD file that specifies the ligand and a pdb file that contains the protein as input. The software then uses simple run directives to automatically set up the energy computation and sampling - making it easier to run than docking since no pre-computed grids are required.

Unlike FEP methods, Iron does not require the assumption of an initial pose. While Iron can use constraints to bias the sampling to retain important interactions, these are not required. In routine calculations, Iron searches exhaustively across a designated binding site to identify the lowest energy pose and conformation. The absence of constraints facilitates the identification of novel binding modes, evaluation of diverse chemical series, and 'target hopping' to new protein targets.

### Key Advantages

- Easy set up and fast calculations for studying multiple ligands
- Unbiased sampling and flexible protein for efficient hopping to new chemical series and targets
- Explicit accounting for solvent to maximize ligand efficiency
- FEP-level accuracy to move designs from computer to synthesis with increased confidence.

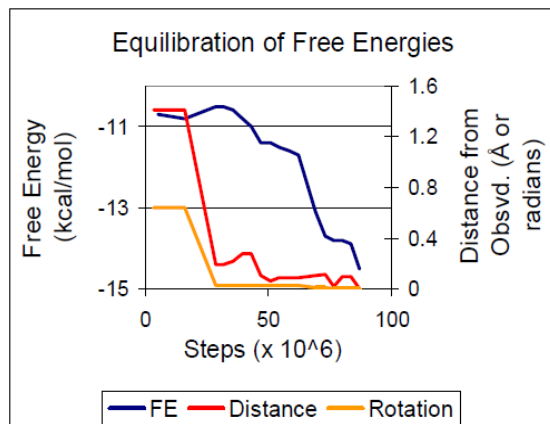
### *The most flexible, and easiest to use free-energy calculation available.*

- The most flexible and easy to use free energy calculation available.
- Fast and readily automated for simulation of large numbers of potential ligands.
- Most simulations run in under two hours, even with five or more rotatable bonds. Calculations match the pace of Medicinal Chemistry.
- Computes binding free-energies of water in the binding site to guide ligand design and correct for protein solvation energy.
- Includes protein flexibility to evaluate a wider range of chemical structures. Simultaneously optimize for potency, in vivo properties, and synthesizability.
- Distinguish active from inactive with >90% confidence.
- Novel technologies in Iron are patent pending

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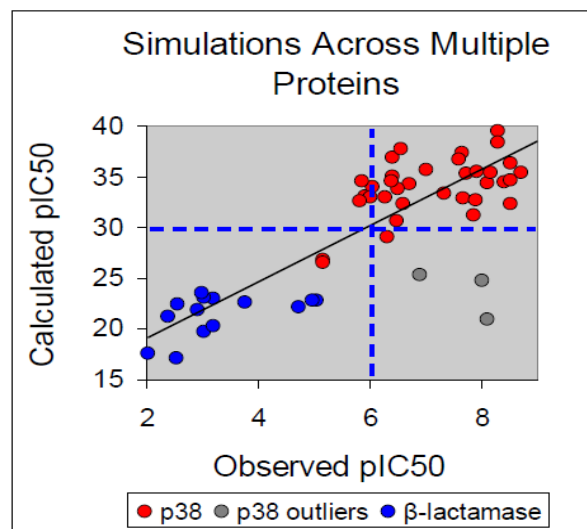
## Binding Free Energies Computed For Three Diverse Systems Demonstrate Flexibility and Accuracy.



The graph at left shows the convergence properties of the Iron free energy computation. The red and yellow lines show that structural convergence to the crystallographic pose is achieved comparatively rapidly. Convergence to the lowest free energy is significantly slower, however. This is due to the sensitivity of the enthalpy to comparatively small changes in pose and to the requirement to integrate over an unbiased sampling of poses to compute the entropy.

These results explain in part why docking methods are historically unreliable for predicting binding affinity. Calculations that are focused on convergence of structure may be concluded prematurely for identifying the correct binding enthalpy. In addition, of course,

docking methods are not well suited for computing the entropy term. In order to show the generality of the method, two diverse systems<sup>1,2</sup> spanning 10 kcal/mol in binding energy were computed without constraints. Each of the 51 ligands was sampled in all possible poses in the binding site. The result, shown in the graph to the right, indicates that the calculations are consistent for diverse proteins and for ligands with diverse structures spanning a wide potency range. Based on the trend line, the standard error for predicting the observed pIC50 is 0.9 log units (1.2 kcal/mol) without correction for ligand solvation. This level of accuracy is sufficient to distinguish actives from inactives with ~90% confidence (based on simulated data). To illustrate, if our goal were to identify and synthesize only compounds with nM potency, then only those compounds that scored above the dashed blue line in the figure would be synthesized; and we would miss only 3 compounds of interest (grey circles) while correctly classifying 26 compounds.



*Technologies used in Iron are patent pending, US 12/626,911.*

### System Requirements

- Sun JAVA run-time system (JRE), which is available for Linux, Solaris, Windows (2000/2003/2008/XP/Vista) Mac OS X and other systems.
- Reads SD file and PDB format files for input,

The system is delivered as a self-contained "jar" file with all software, and configuration files packaged into a single file. This mechanism makes installation very easy and flexible. The use of the JAVA language allows you to get exactly the same result on any type of computer.