Predicting Absolute Protein-Ligand Binding Affinities of Charged Molecules Using Molecular Dynamics Free Energy Calculations

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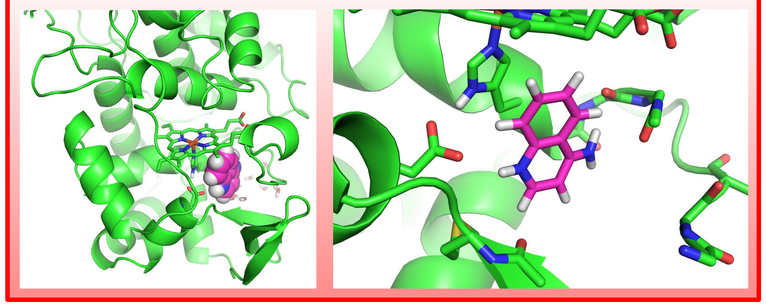
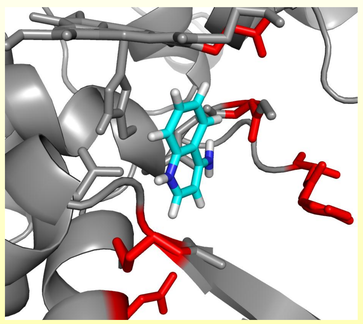
The Test: Cytochrome C Peroxidase W191G-Gateless

A model binding site for small cations

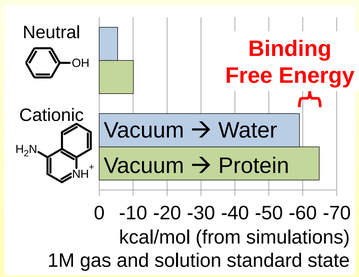


The Obstacles

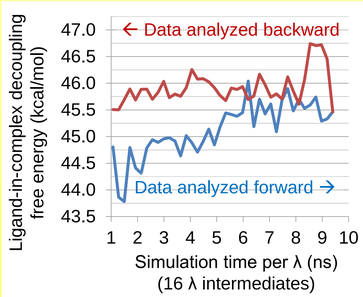
Protein flexibility



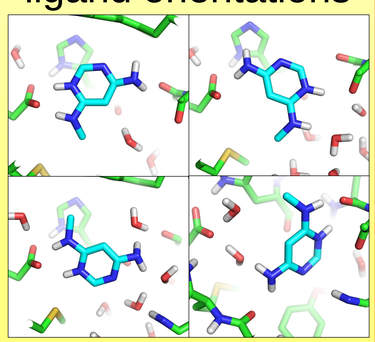
Strong electrostatic interactions



Convergence of ΔG estimates from simulations

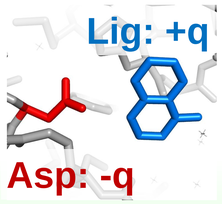


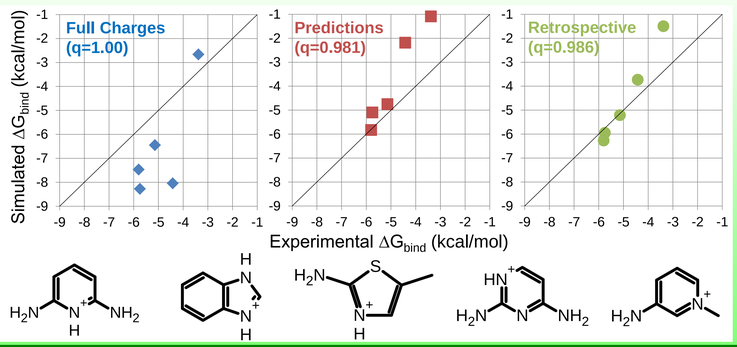
Many possible ligand orientations



Blind Test #1 Results

Using smaller, non-integral net charges for the ligand and aspartate improves accuracy and rank-ordering.

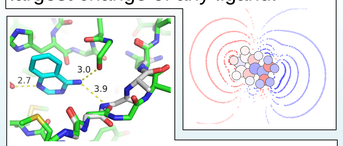




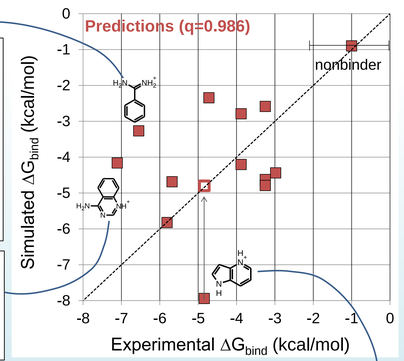
Blind Test #2 Results

More challenging ligands identify likely problems with simulations

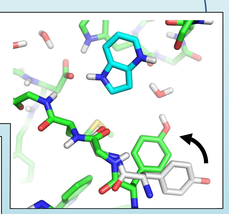
Changing AM1-BCC charges to QM ESP cuts this error by half, the largest change of any ligand.



Conformational change observed in crystal structure (green) is sampled – but not favored – in simulations. (Apo struct. in gray)



Protein adopted alternate conformation (green) during ligand equilibration, and the free energy cost of the change was never accounted for, causing binding to appear too favorable. (Apo struct. in gray)



1. Rosenfeld RJ, Hays AMA, Musah RA, Goodin DB. (2002) Excision of a proposed electron transfer pathway in cytochrome c peroxidase and its replacement by a ligand-binding channel. *Protein Science* 11, 1251

What have we learned?

1. Prediction quality is very modest

Affinity

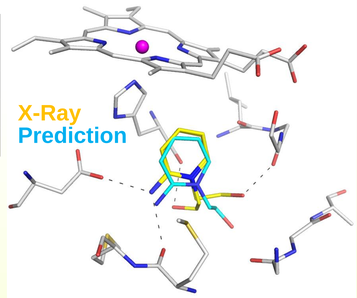
Specific failures (left) lead to poor correlation, but modest ranking. QM ESP charges improve results.

	RMS	R^2	Tau
Blind Prediction	1.85	0.07	0.26
AM1-BCC Best	1.50	0.14	0.34
" (cations only)	1.55	0.18	0.33
QM ESP Best	1.37	0.36	0.55

RMS in kcal/mol. RMS and R^2 exclude the nonbinder, which is correctly predicted as the weakest ligand by all methods
QM ESP data includes only cations.'Best' = scaled and corrected for Tyrosine flip.

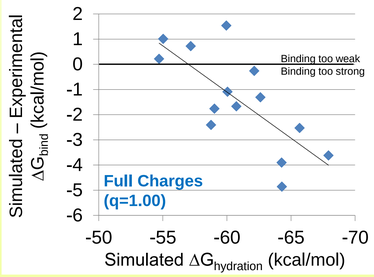
Geometry

5 of 5 new binders < 1 Å RMSD
9 of 12* binders have correct contacts favored in simulations

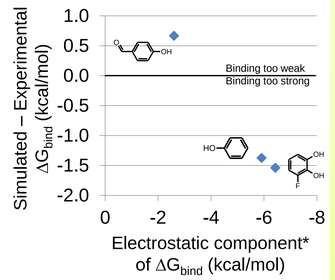


2. Systematic error in unscaled simulations overstabilizes binding

Error in unscaled results for cations correlates with polarity



Binding of neutrals is overstabilized as well



3. More detailed models improve agreement with experiment.. so far

Moving from semi-empirical (AM1-BCC) partial charges to QM ESP charges improves overall agreement, but affinity is still overestimated unless charges are scaled..

Can we understand and fix the overall systematic error?