Absolute Binding Free Energy Calculations in Charged Model Systems: Cytochrome C Peroxidase W191G-Closed and W191G-Gateless

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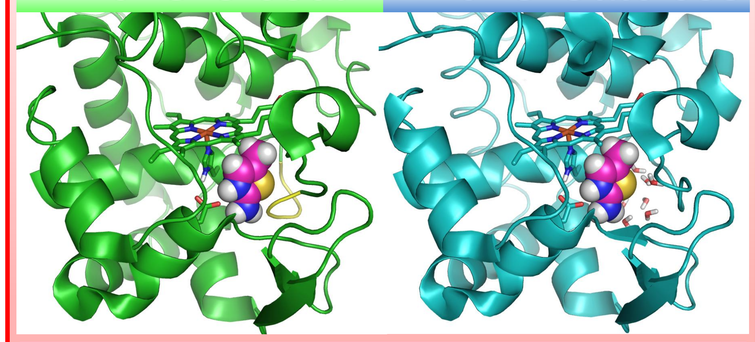
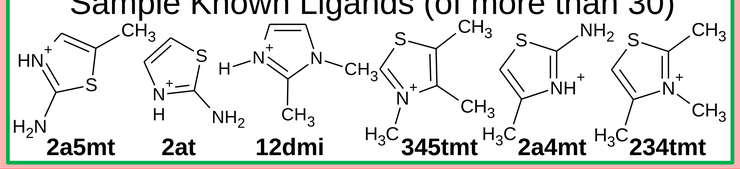
Charged Model Systems

Two different CCP W191G proteins that bind small cations

W191G-Closed **W191G-Gateless**



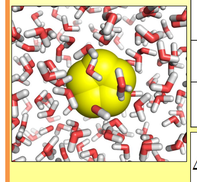
Sample Known Ligands (of more than 30)



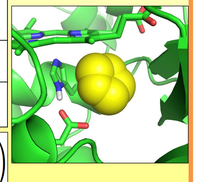
2a5mt **2at** **12dmi** **345tmt** **2a4mt** **234tmt**

How do the binding sites differ electrostatically?

Use Born relation and a probe molecule to determine the effective dielectric constant of Closed and Gateless in comparison to water



Site	ϵ	ϵ dehyd.	Potential (kT/q)
Closed	3.75	2.33	-149
Gateless	3.70	1.52	-139

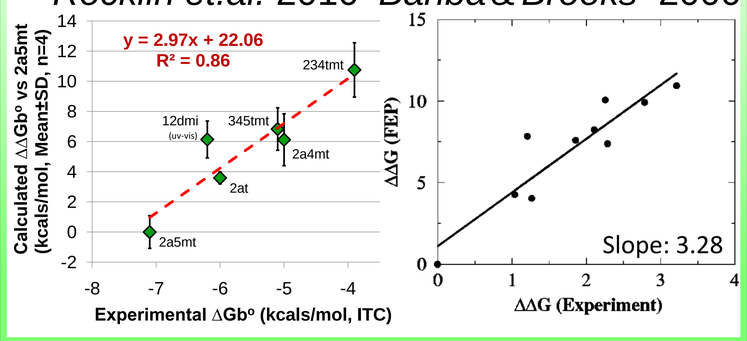


$$\Delta G_{\mathrm{rlx}} = \frac{1}{2}\frac{\partial^2 G}{\partial q^2} = -\frac{q^2}{4\pi\epsilon_0}\frac{1}{2R}\left(1-\frac{1}{\epsilon}\right)$$

Surprisingly, ϵ's are very close, but more relaxation comes from water in Gateless

Relative calculations produce steep slopes across methods

Rocklin et.al. 2010 *Banba & Brooks*[1] 2000



Two groups with different force fields, ligand parameters, and sampling methods both get ΔΔG wrong in CCP W191G by a consistent factor of 3!

Absolute calculations in Gateless also steeply sloped

Both neutral and charged ligands are predicted to bind, but with the same slope that makes the strongest ligands too strong

Ligand	q	ΔGb,comp (Gateless)	ΔGb,exp (Closed) [2]	ΔGb,exp (Gateless) [3]
3-fluorocatechol	0	-3.5	-2.9	Binds
phenol	0	-5.8	-3.3	Binds
234tmt	+1	-9.4	-3.9	-3.6
2a4mt	+1	-13.1	-5.0	No data
345tmt	+1	-11.2	-5.1	-4.5
2at	+1	-14.3	-6.0	No data
12dmi	+1	-15.0	-6.2	-3.6?
2a5mt	+1	-17.8	-7.1	No data

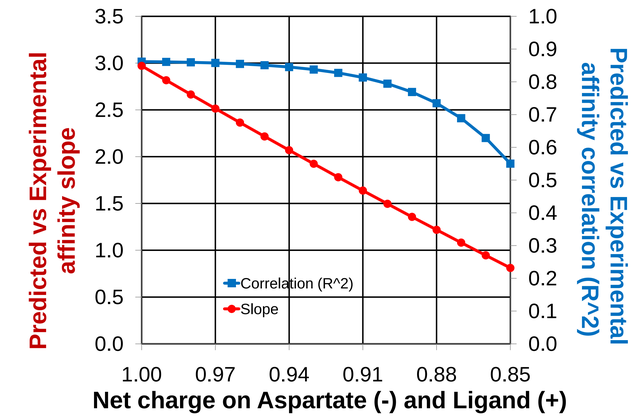
1. Banba S, Brooks CL III. (2000) Free energy screening of small ligands binding to an artificial protein cavity. *J. Chem. Phys.* 113, 3423

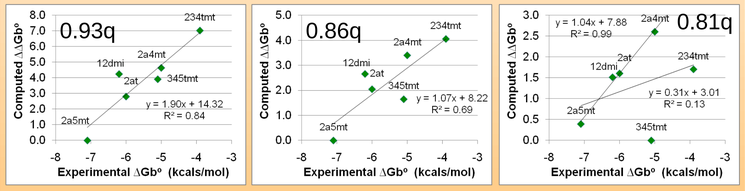
2. Musah RA, Jensen GM, Bunte SW, Rosenfeld RJ, Goodin DB. (2002) Artificial Protein Cavities as Specific Ligand-binding Templates. *J. Mol. Biol.* 35, 845

3. 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

Why do affinity calculations overpredict ΔΔG?

Reducing the net charge on the bound ligand and Asp232 lowers the slope





Asp232 feels a much smaller potential than other Asps and would be less polarized