Using Free Energy Perturbation Calculations to Model the Mutation of $LeuT_{Aa}$ and mDAT Residues that Bind TCAs

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Advisor: Dr. Jeffry Madura

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Free Energy Perturbation Calculations?

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Free Energy Perturbation (FEP) Calculations

- Can be used to predict manifold biological phenomena
  - pKa
  - change in binding energy due to mutagenesis
  - solvation energy
  - protein-ligand binding
- Usually the most accurate way to calculate any of the above

The Zwanzig Equation

$$\Delta G(A \rightarrow B) = G_B - G_A = -k_B T \ln \left\langle \exp\left(-\frac{E_B - E_A}{k_B T} \right) \right\rangle_A$$

Zwanzig, R.W. **1954**. *J. Chem. Phys.* 22; 8: 1420-1426.

Implementation in NAMD 2.7b1

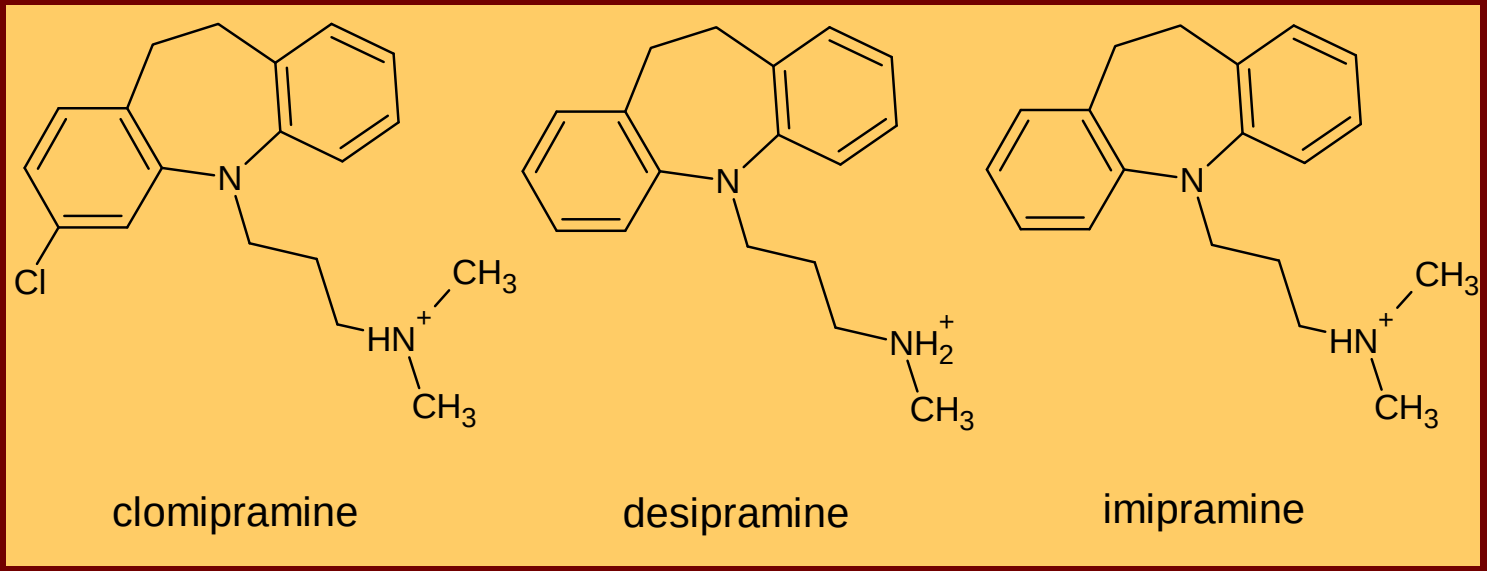
$$\Delta G(i \rightarrow i + \Delta i) = -k_B T \sum_{i=1}^{N} \ln \left\langle \exp\left(-\frac{\mathrm{H}_B(x, p_x; \lambda_{i+\Delta\lambda}) - \mathrm{H}_A(x, p_x; \lambda_i)}{k_B T} \right) \right\rangle_i$$

Parameterizing a Novel Molecule

- Determines how a force field (e.g., CHARMM) will treat the molecule
  - Atomic Partial Charges
  - Bond lengths and angles
  - Spring Constants for bonds and angles
    - $K_b(b-b_0)^2$
  - Energy function for rotation around dihedral bonds
- Atomic Partial Charges were assigned via the OPLS-AA force field
- Remaining parameters were set via (educated?) guesses

LeuT and TCA Binding

- The crystal structure of $LeuT_{Aa}$ was reported in 2005.
- $LeuT_{Aa}$ was also crystallized bound to several tricyclic antidepressants (TCAs)



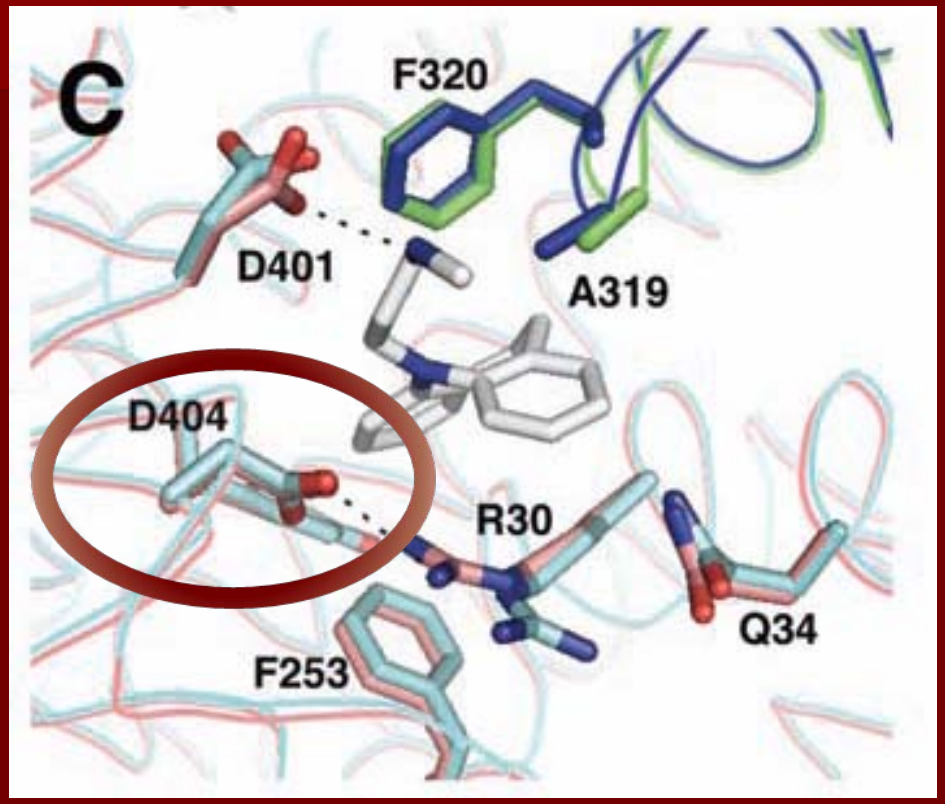
Yamashita, A. et al. **2005**. *Nature*. 437: 215-223.
Zhou, Z. et al. **2007**. *Science*. 317: 1390-1393.
Singh, S.K. et al. **2007**. *Nature*. 448: 952-956.

Varying Steps/Window

Mutated Residue: LeuT Asp 404 → Ala	
Steps/Window	ΔG_{D404A} (kcal/mol)
100,000	114.4
200,000	115.2
400,000	119.7

Calculations performed in vacuum

The Mutation



Aspartate 404 mutated to alanine with clomipramine bound

Zhou, Z. et al. **2007**. *Science*. 317: 1390-1393.

Modeling Ligand Binding with FEP Calculations

$$LeuT + TCA \xrightarrow{\Delta\Delta G_{bind}} LeuT : TCA$$

$$LeuT : TCA \xrightarrow{\Delta G_1} LeuT : \bullet$$

$$TCA \xrightarrow{\Delta G_2} \bullet$$

Modeling Ligand Binding with FEP Calculations

$$LeuT + TCA \xrightarrow{\Delta\Delta G_{bind}} LeuT : TCA$$

$$LeuT : \bullet \xrightarrow{-\Delta G_1} LeuT : TCA$$

$$TCA \xrightarrow{\Delta G_2} \bullet$$

$$\therefore \Delta\Delta G_{bind} = \Delta G_2 - \Delta G_1$$

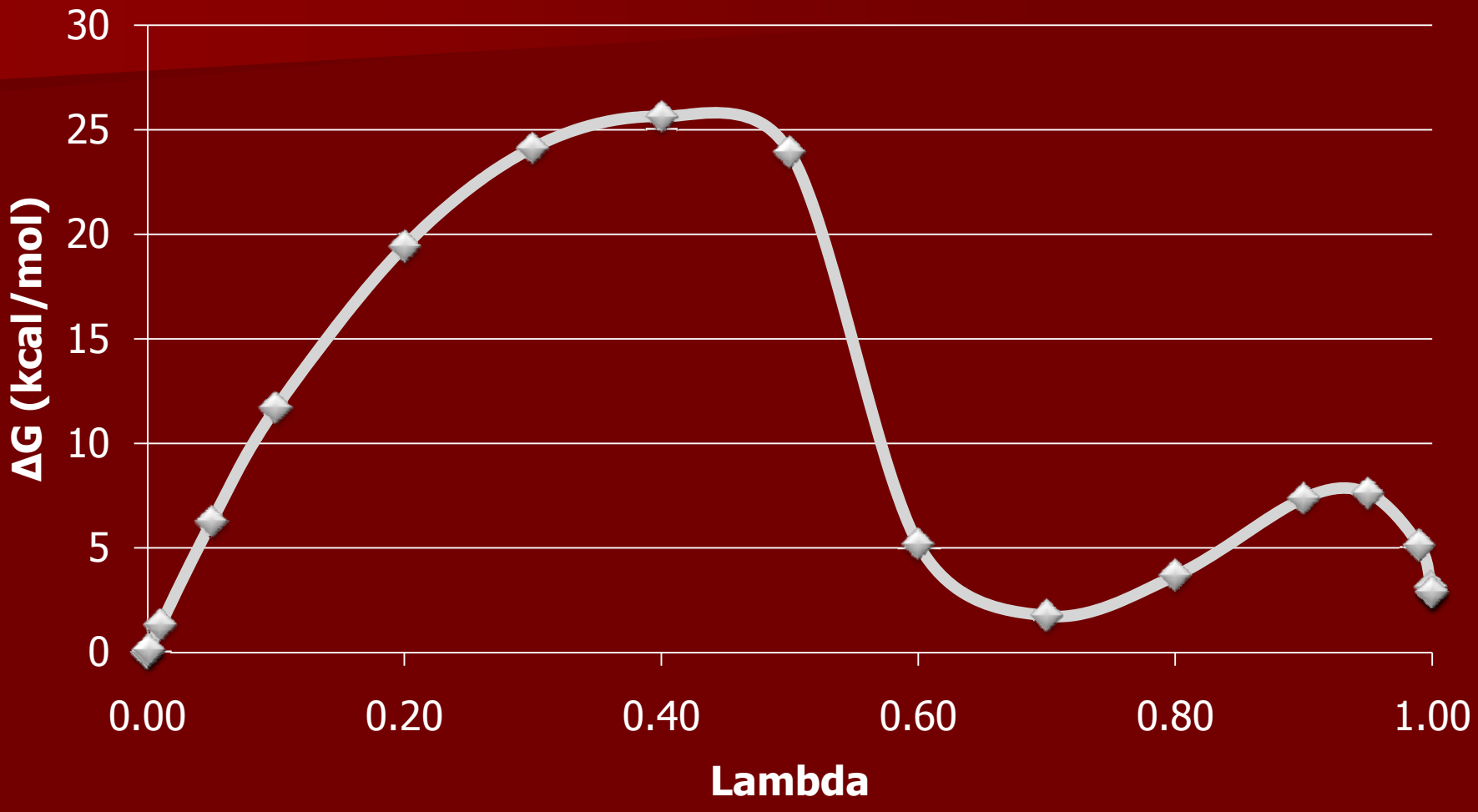
Initial TCA Binding Results

FEP Calculations of Aqueous $\Delta\Delta G_{binding}$ Results				
TCA (Complex)	$\Delta G_{TCA \rightarrow \sim}$ (kcal/mol)	$\Delta G_{LeuT:TCA \rightarrow LeuT:\sim}$ (kcal/mol)	ΔG_{bind} (kcal/mol)	ΔG_{exp} (kcal/mol)
clomipramine	2.9	15	-12	-4.9 ± 0.09
imipramine	35	12	23	-3.7 ± 0.08

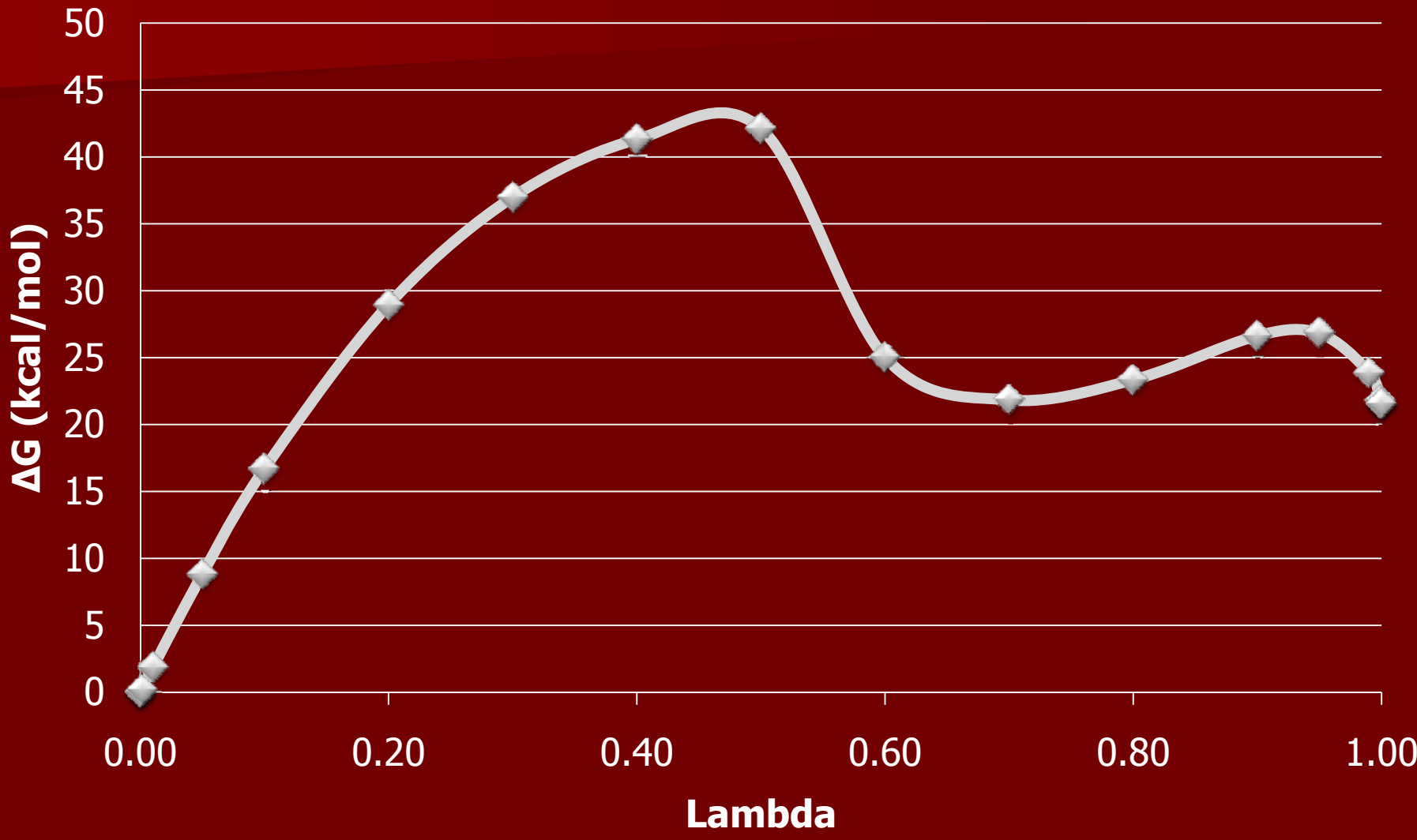
- $\Delta\Delta G_{binding} = RT\ln(IC_{50})$ for non- or un-competitive binding

Experimental values from Singh, S.K. et al. **2007**. *Nature*. 448: 952-956. (supplementary material)

Clomipramine → ~



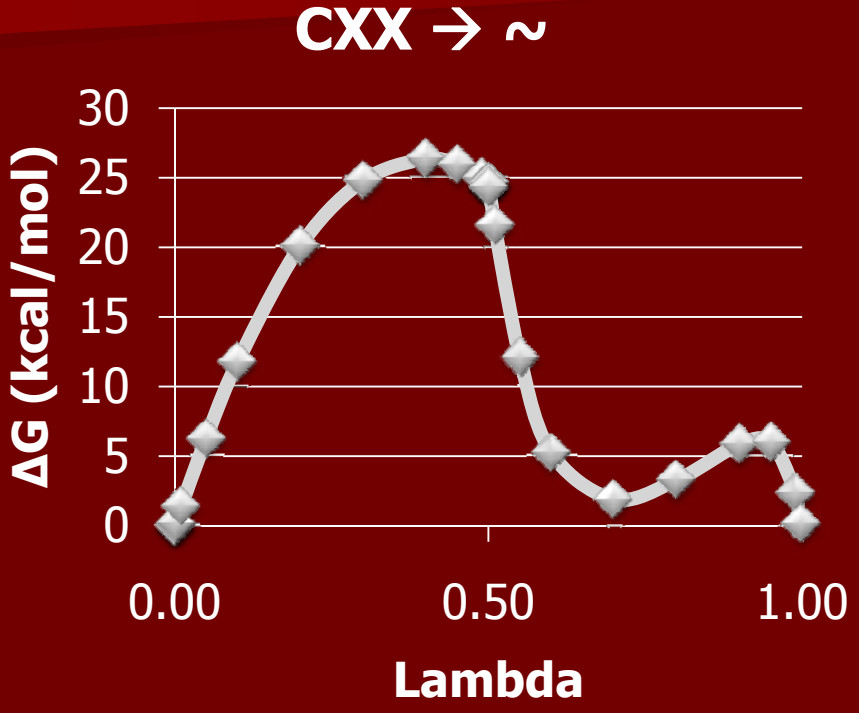
Desipramine → ~

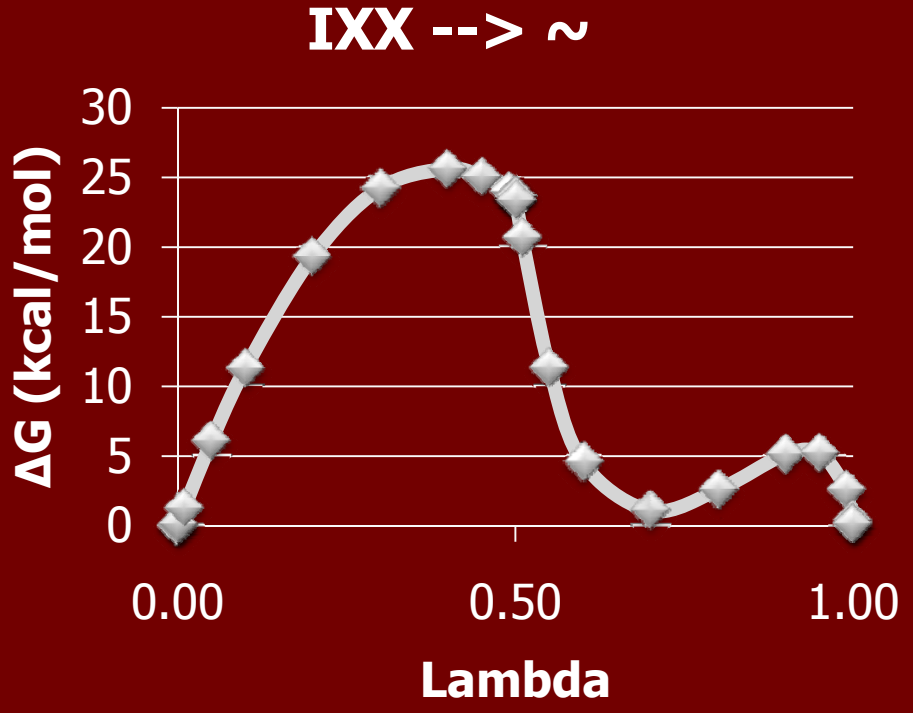


Potential Sources of Error

- Inadequate sampling near $\lambda = 0.5$
  - More trials run with better sampling (still waiting for results from supercomputer)
- OPLS charges incompatible with force field
  - Performed ab initio calculations to compare to OPLS charges
- FEP calculations incorrectly implemented
  - Ran several variations of FEP calculations using decane (with OPLS charges and guessed parameters)
  - Prepared a set of 14 calculations with CHARMM-parameterized molecules (will run when supercomputer wakes up)

TCA → ~ with Better Sampling

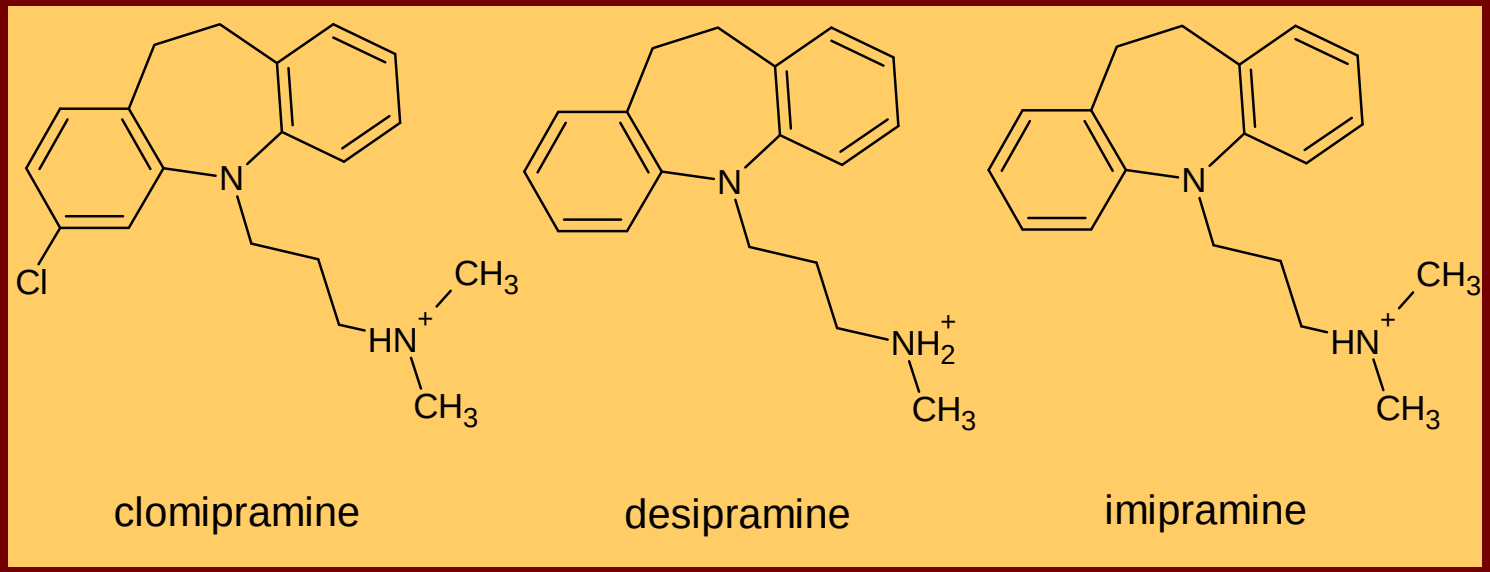




100K steps / window, 39 windows
Electrostatics decoupled λ = 0 – 0.5, van der Waals decoupled λ = 0.5 – 1.0

Comparison of TCA → ~ Calculations

	25 Windows (kcal/mol)	39 Windows (kcal/mol)
clomipramine	2.86	-0.0865
imipramine	35.0	0.184
desipramine	21.6	21.9

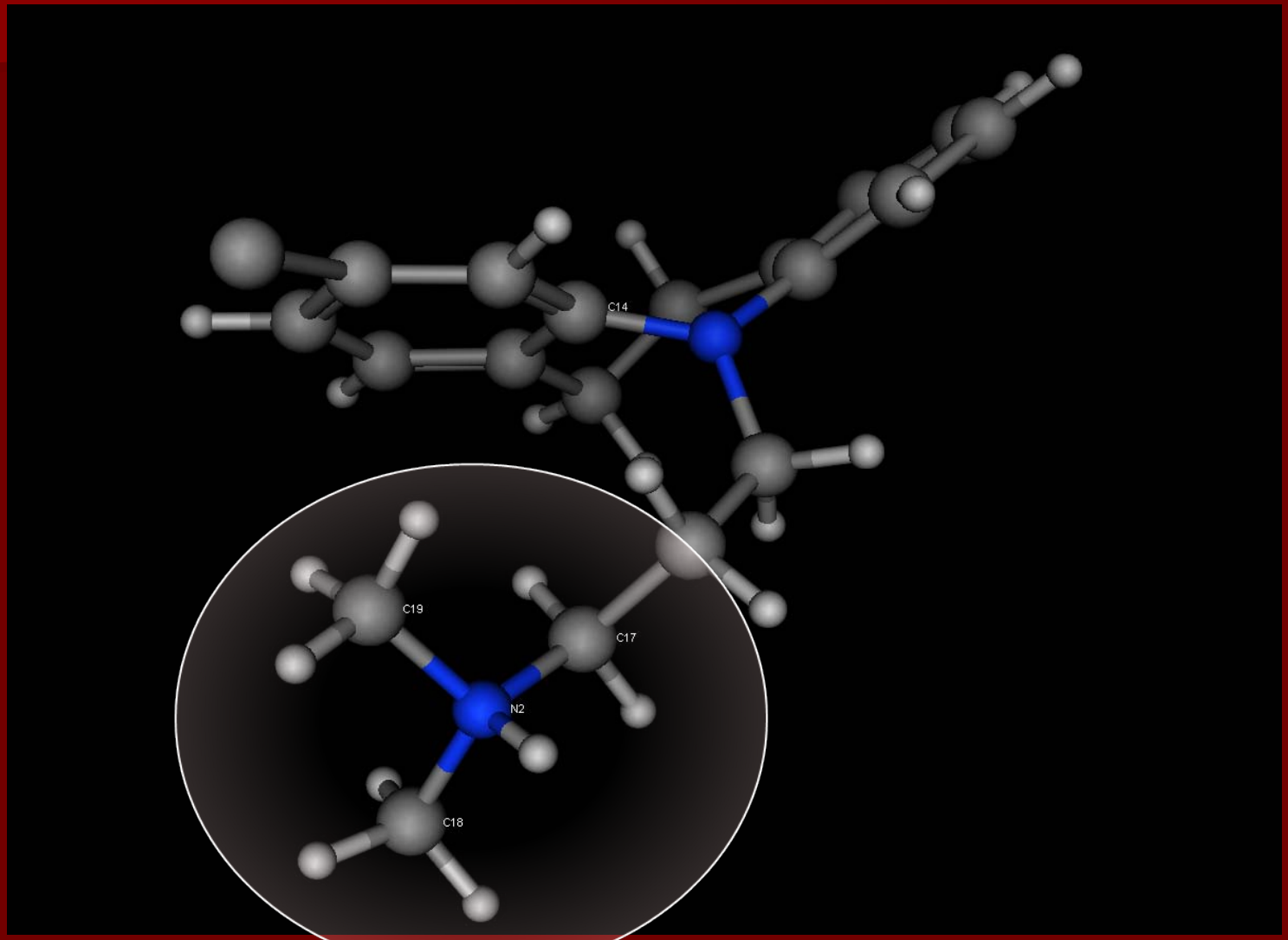


Alternative Charges

		Minimized Structure		XRD Structure	
	OPLS-AA	MSK	ChelpG	MSK	ChelpG
Total Charge	1.00	0.992	1.001	0.998	1.00
Abs. Dev / N	--	0.090	0.080	0.112	0.088
Most significant deviations					
Atom	Charges				
N2	-0.260	0.003	0.005	0.103	-0.058
C14	0.123	0.267	0.336	0.126	0.214
C17	0.190	0.319	0.124	-0.216	0.075
C18	0.130	-0.359	-0.217	-0.387	-0.262
C19	0.130	-0.327	-0.152	-0.438	-0.194

Calculated in Gaussian 03 at the HF/6-31G(d) level of theory; the minimized structure contained no imaginary frequencies.

Most Significant Deviations



Determining $\Delta\Delta G_{solv}$

$$A_{vac} \xrightarrow{\Delta\Delta G_{solv}} A_{aq}$$

$$\bullet_{aq} \xrightarrow{-\Delta G_1} A_{aq}$$

$$A_{vac} \xrightarrow{\Delta G_2} \bullet$$

$$\Delta\Delta G_{solv} = \Delta G_2 - \Delta G_1$$

Results for FEP Calculations on Decane

				Aqueous			
			Decouple	ON		OFF	
		Fixed?		Yes	No	Yes	No
	Boundary		Results (kcal/mol)	0.20	-0.058	-0.45	-4.9
Vacuum	Periodic	Yes	-0.27	-0.47	-0.21	0.18	4.3
		No	-2.6	-2.8	-2.5	-2.1	2.3
	None	No	-2.3	-2.5	-2.2	-1.9	2.6

Experimental decane ΔG_{solv}= 3.16 kcal/mol

Using Parameterized Molecules to Test FEP Methods

Testing FEP Method with Parameterized CHARMM Test Set

Compound	ΔG_{solv} (kcal/mol)		Compound	ΔG_{solv} (kcal/mol)	
	Calc.	Exp.[5]		Calc.	Exp.[5]
acetic acid	-12	-6.69	methanol	-4.0	-5.10
benzene	-0.82	-0.86	methylamine	-2.7	-4.55
butane	1.8	2.07	N-methylacetamide	-12	-10.00
ethanol	-6.0	-5.00	pentane	1.1	2.32
ethane	2.2	1.83	phenol	-7.8	-6.61
ethanethiol	-0.98	-1.10	propane	2.2	1.96
methanethiol	-0.35	-1.20	protene	0.80	1.32

Average Deviation from experiment: 1.2 kcal/mol

Exp. Values: Rizzo, R.C. et al. **2006**. *J. Chem. Theor. Comp.* 2: 128-139.

Future Work

- Continue to refine TCA CHARMM parameters in a more systematic manner
  - Use new parameters to perform calculations that can be compared to experimental results (e.g., pKa prediction)
- Investigate alternative methods for implementation of FEP calculations into NAMD, including those that can separate electrostatic decoupling from van der Waals
- Use proper TCA parameters to perform FEP calculations that simulate the mutation of an inhibitor-binding residue and compare these results with mutagenesis studies. This data can be used to refine the computational model of TCA binding to both $LeuT_{Aa}$ and mammalian sodium symporters (e.g., DAT).

Acknowledgements

- Dr. Jeffry Madura, Duquesne University
- Kalyan Immadisetty, Ph.D. candidate, Duquesne University
- The BBSI administrators
- All the cool BBSI students, and Victor Rusu
- NIH/NSF