Probing the Inhibitor Binding Site of Neurotransmitter Symporters Using FEP Calculations

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Abstract

Neurotransmitter Sodium Symporters, a family that includes dopamine and serotonin transporters, are the target of several inhibitors, including the tricyclic antidepressants desipramine, clomipramine, and imipramine. Crystal structures of the homologous bacterial transporter, LeuT, have been solved with these inhibitors bound. Parameterization of the inhibitors in the CHARMM force field was attempted, but the results of free energy perturbation (FEP) calculations using the parameters compared poorly with experimental results. Several alternative methods of FEP calculations were explored, and additional sets of parameters are suggested for further refinement.

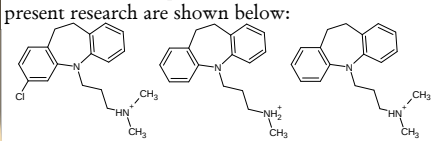
Introduction

Free energy perturbation (FEP) calculations can be used to predict the free energy change that accompanies manifold biochemical phenomena, including protein–ligand binding, mutation of a residue, and solvation of a molecule. The calculations use the Zwanzig equation[1] to estimate the change in energy between two states:

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

The calculation can be separated into several windows, with the results summed to determine the total free energy change. The general extent parameter (λ) defines the windows, which can be calculated in parallel.

The tricyclic antidepressants (TCAs) that were crystallized in complex with LeuT[2] and used in the present research are shown below:



clomipramine desipramine imipramine

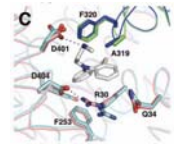
Methods

- Modeling a D404A mutation in LeuT:clomipramine complex
  - 100K, 200K, and 400K steps/window
  - 25 windows, small Δλ at beginning and end of calculation
  - System in vacuum
- Aqueous $\Delta\Delta G_{binding}$ calculations
  - 100K steps/window, 25 windows
  - values using the following equation:

$$\Delta G_{binding} = RT \ln(IC50)$$

- Alternative atomic charges for clomipramine
  - Calculated at the HF/6-31G(d) level of theory
  - Both MSK and ChelpG methods employed
- Energy of solvation FEP calculations
  - 100K steps/window, 39 windows
  - Better sampling around λ = 0.5, where electrostatics are fully decoupled and van der Waals begin to be decoupled
  - Performed on decane and parameterized CHARMM test set

•All FEP calculations performed in NAMD 2.7b1[4] using periodic boundary conditions, Langevin dynamics, and TIP3P water when appropriate.



The LeuTAa clomipramine binding pocket, from ref. (3)

$$LeuT + TCA \xrightarrow{\Delta\Delta G_{bind}} LeuT:TCA$$
$$LeuT:\bullet \xrightarrow{-\Delta G_1} LeuT:TCA$$
$$TCA \xrightarrow{\Delta G_2} \bullet$$
$$\Delta\Delta G_{bind} = \Delta G_2 - \Delta G_1$$

Method for determining $\Delta\Delta G_{binding}$

$$A_{vac} \xrightarrow{\Delta\Delta G_{solv}} A_{aq}$$
$$\bullet_{aq} \xrightarrow{-\Delta G_1} A_{aq}$$
$$A_{vac} \xrightarrow{\Delta G_2} \bullet$$
$$\Delta\Delta G_{solv} = \Delta G_2 - \Delta G_1$$

Method for determining $\Delta\Delta G_{solv}$

Results

Mutated Residue: LeuT Asp 404 → Ala

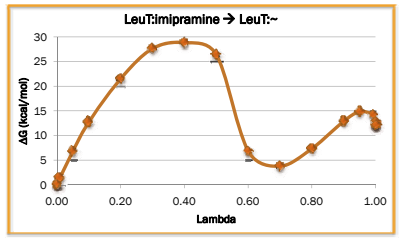
Steps/Window	ΔG_{D404A} (kcal/mol)
100,000	114.4
200,000	115.2
400,000	119.7

FEP Calculations of Aqueous $\Delta\Delta G_{binding}$ Results

TCA	$\Delta G_{TCA\rightarrow\sim}$ (kcal/mol)	$\Delta G_{LeuT:TCA\rightarrow LeuT:\sim}$ (kcal/mol)	ΔG_{bind} (kcal/mol)	ΔG_{exp}[2] (kcal/mol)
clomipramine	2.9	15	-12	-4.9 ± 0.09
imipramine	35	12	23	-3.7 ± 0.08

Alternative Atomic 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



Testing Various FEP Methods with Decane (ΔG_{solv} = 3.16)

		Decouple		Aqueous			
				ON		OFF	
	Boundary	Fixed?		Yes	No	Yes	No
			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

Testing FEP Method with Parameterized CHARMM Test Set

Compound	ΔG_{solv} (kcal/mol) Calc.	Exp.[5]	Compound	ΔG_{solv} (kcal/mol) 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

Conclusions

- 100K steps/window or fewer may be sufficient to get reliable FEP results
- Alternative TCA atomic charges would most significantly affect the distribution in the charged tails
- FEP calculations may be most accurate when the all molecules are unfixed and all atoms are coupled to the simulation

Future Research

- Continue to refine CHARMM parameters for TCAs by using various sets 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

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