Sensitivity in predicted relative binding free energies from incremental ligand changes within a model binding site

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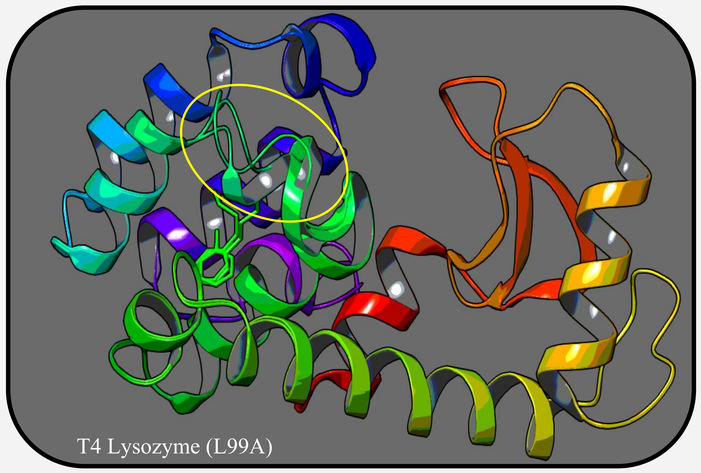
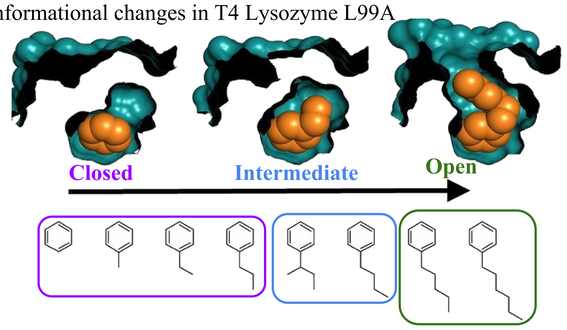
ABSTRACT - Despite innovations in sampling techniques for molecular dynamics (MD), reliable prediction of protein-ligand binding free energies from MD remains a challenging problem, even in well studied model binding sites like the apolar cavity of T4 Lysozyme L99A.[1] In this study, we model recent experimental results that show the progressive opening of the binding pocket in response to a series of homologous ligands.[2] Even while using enhanced sampling techniques, we demonstrate that the predicted relative binding free energies (RBFE) are still highly sensitive to the initial protein conformational state. Particularly, we highlight the importance of sufficient sampling of protein conformational changes and possible techniques for addressing the issue.



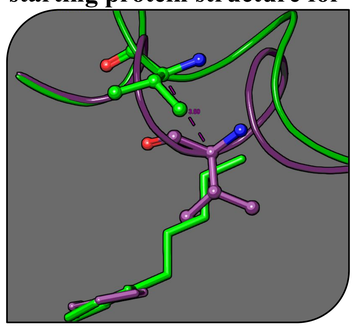
BACKGROUND[2] - A series of related ligands gradually induce conformational changes in T4 Lysozyme L99A



OBJECTIVES

- Model and capture protein conformational changes in the T4 Lysozyme L99A apolar cavity
- Assess convergence of relative binding free energy calculations involving modest amounts of protein conformational change
- Demonstrate how modest ligand changes can induce conformational changes in "rigid" proteins which pose significant sampling challenges

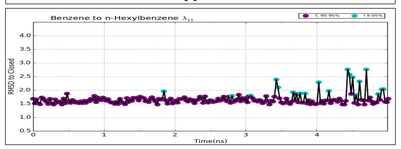
RESULTS - Calculated free energies significantly depend on the starting protein structure for "large" perturbations



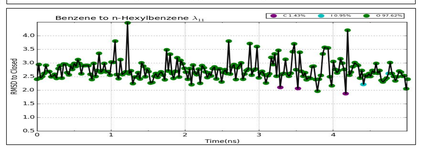
Closed vs Open: Calculated ΔΔG (kcal/mol)				
Lig 1	Lig 2	Closed	Open	*C-O Diff*
benzene	n-hexyl	4.1	-0.6	**4.7**
toluene	n-hexyl	2.9	-1.6	**4.5**
ethyl	n-hexyl	3.6	-0.8	**4.4**
n-propyl	n-hexyl	5.9	0.1	**5.7**

Protein gets trapped in its starting conformation, leading to different predictions due to sampling problems

Start from Closed, trapped in Closed: **ΔΔG = 4.1**

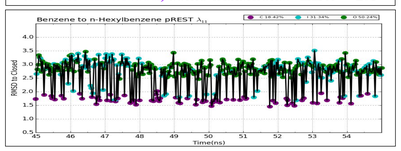


Start from Open, trapped in Open: **ΔΔG = -0.6**

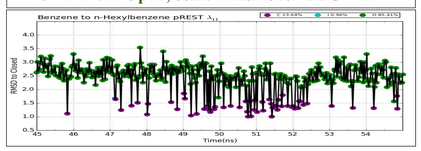


By simulating 10x longer and modifying the REST region, we see a reduction in the discrepancy from populating both states

Start from Closed, some transitions: **ΔΔG = 2.1**



Start from Open, some transitions: **ΔΔG = 1.4**



Total C-O RMSE after 55ns simulations and REST region modifications

C-O RMSE: **4.0** kcal/mol → C-O RMSE: **0.6** kcal/mol

Figure 1. Correlation between relative binding free energies calculated from closed and open simulations.

RMSE = 0.57, N=8

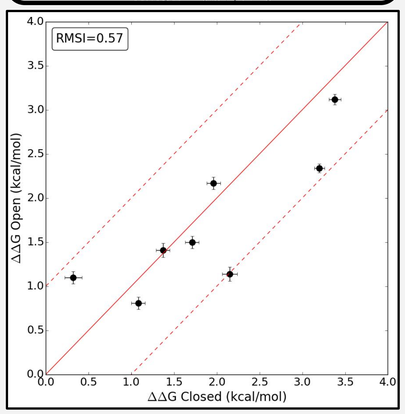
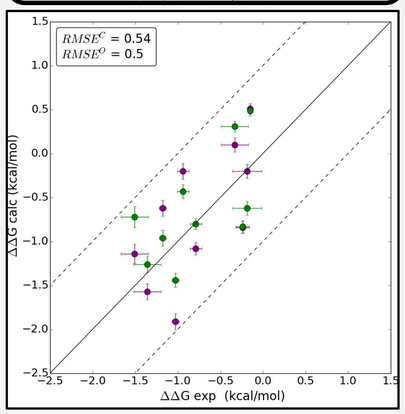
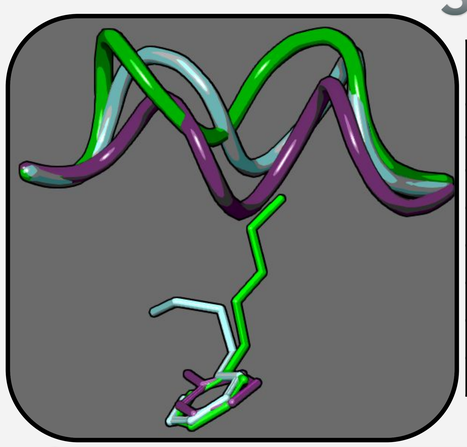


Figure 2. Correlation between experimental binding affinities versus relative binding free energies, from both closed and open simulations.

RMSE = 0.54, N=10





Ligand	ΔGexp (kcal/mol)
benzene	**-5.19**
toluene	**-5.52**
ethylbenzene	**-5.76**
n-propylbenzene	**-6.55**
sec-butylbenzene	N/A
n-butylbenzene	**-6.70**
n-pentylbenzene	N/A
n-hexylbenzene	N/A

APPROACH - *FEP/REST[3] with OPLS3[4] forcefield parameters*

- From experiment, we find bigger ligands seem to bind better but induce more conformational change in the protein.
- In our procedure, we start simulations from either the protein **closed** or **open** conformation.
- Then, we perform alchemical transformations to ligands that would induce a protein conformational change.
- If needed, we include protein residues in the REST region to facilitate faster transitions between conformational states.
  - Finally, we attempt to converge to the same calculated free energy and eliminate the dependence on the initial protein conformation.

CONCLUSIONS

- Special attention should be used when performing binding free energy calculations where regions of flexibility surround the binding site.
- Even with modern enhanced sampling methods, capturing even moderate and localized protein structural rearrangements still poses a problem within the standard 5ns time frame of free energy calculations.
- Without prior knowledge of protein-ligand discrete conformational states, we can arrive at very different predictions in relative binding free energies that significantly depend on the initial protein structure.

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