Comparing Receptor-Ligand Restraint Schemes for Alchemical Absolute Binding Free Energy Calculations

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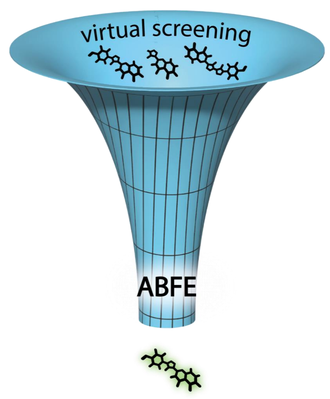
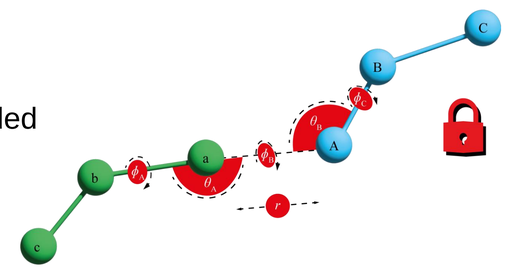
Alchemical absolute binding free energies are of increasing interest in drug discovery

- Alchemical absolute binding free energy (ABFE) calculations allow the rigorous calculation of binding free energies of **diverse molecules**, and show promise to deliver significant value in drug discovery campaigns[1]
- **Receptor-ligand restraints** are required to **prevent sampling issues**

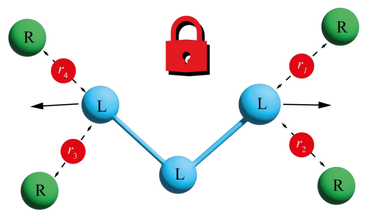


Several varieties of restraints are available

Boresch[2]
- ✓ Simple analytical correction
- ✗ Limited in the extent of restraint provided
- ✗ Instabilities



Multiple distance restraints[3]
- ✓ Closely mimic native receptor-ligand interactions, potentially enhancing convergence

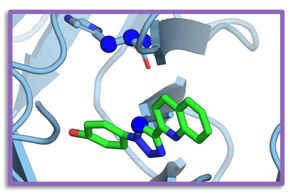


Distance or positional restraint
- ✓ Simple
- ✗ Poorest convergence

Lack of orientational restraints produced erroneously negative free energies

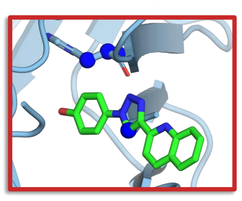
- Up to 4 kcal mol^{-1} more negative than with Boresch
- Likely due to failure to sample alternative orientations over few λ windows

Run 4

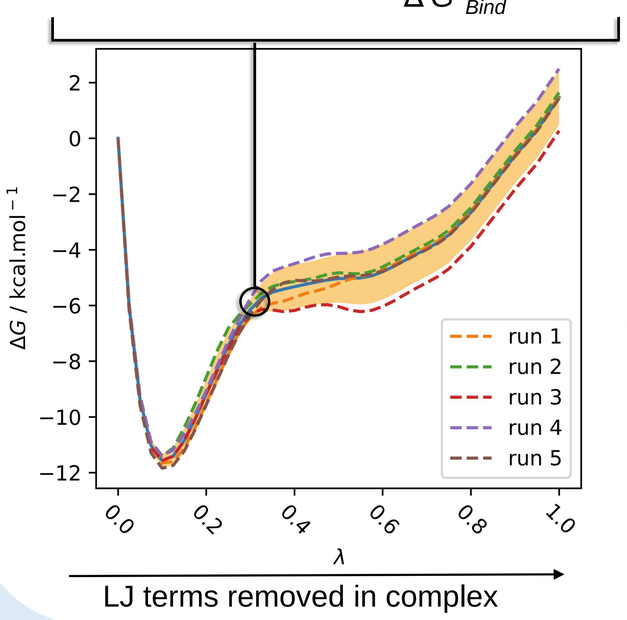


- Stuck in crystal pose orientation
- Largest error

Run 3



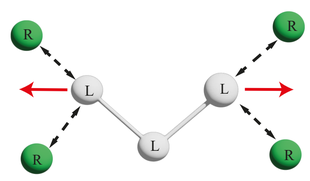
- Exploration of alternative pose
- 2 kcal mol^{-1} improvement in ΔG^{o}_{Bind}



LJ terms removed in complex

Multiple distance restraints can be implemented rigorously

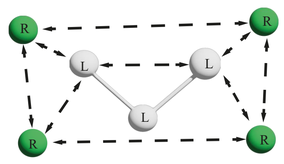
$$\Delta G^{0}_{Release} = k_B T \ln\left(\frac{C^{\circ}}{8\pi^2}\right) + k_B T \ln \int_0^{\infty}\int_0^{\infty}\int_0^{\infty}\int_0^{2\pi}\int_0^{\pi}\int_0^{2\pi} \sin\theta \exp\left[-\beta\left(u(r_x, r_y, r_z, \psi, \theta, \phi)\right)\right] dr_x dr_y dr_z d\psi d\theta d\phi$$



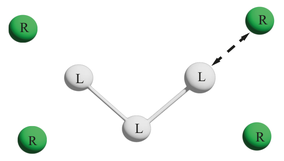
- **Free energy of releasing decoupled ligand cannot be calculated exactly** due to coupling of internal and relative external degrees of freedom

Solutions:

- Rigidify anchor points



- Release all but one restraint[4]



We use MIF / MIF180 as a test system[5]

- Molecular dynamics engine: SOMD (Sire/ OpenMM), 5 replicates, 4 fs timestep with HMR, ff14SB and GAFF2 force fields.
- Restraints: Automated selection of parameters to accelerate convergence via post-processing of 6 ns MD simulation

Equivalent results obtained with Boresch and multiple distance restraints

(Uncertainties reported as 95 % C.I.s)

✓ **Boresch restraints** with different anchor sets generally in good agreement

✓ **Rigorous multiple distance restraint schemes** provide binding free energies in good agreement with Boresch restraints

✗ Performance of **non-rigorous multiple distance restraints** protocol depends on restrictiveness of restraints

✗ **Removal of orientational restraints** results in erroneously negative free energies of binding, likely due to sampling issues at intermediate stages of vanishing

Conclusions

✓ **Rigorous implementations of multiple distance restraints generate answers equivalent to Boresch restraints** but may **offer convergence benefits for larger, flexible ligands**

✗ **Failure to restrain orientation results in erroneously negative free energies of binding** with our protocol

References

[1] Z. Cournia et al., *J. Chem. Inf. Model.*, 2020, **60**, 4153–4169.
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[6] F. Clark, G. Robb, D. Cole and J. Michel, *ChemRxiv*, 2023.