Have Alchemical Free Energy Simulations Come of Age?

New and Remaining Challenges

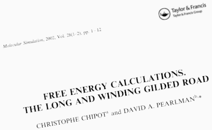
Stefan Boresch

March 18, 2024

Faculty of Chemistry, University of Vienna, Austria

Introduction

History of free energy simulations (FES) — a roller coaster ride



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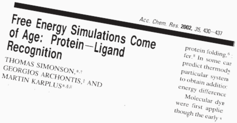
Molecular Simulation, 2002, Vol. 28(1–2), pp. 1–12

FREE ENERGY CALCULATIONS.
THE LONG AND WINDING GILDED ROAD

CHRISTOPHE CHIPOT[a] and DAVID A. PEARLMAN[b,*]



Acc. Chem. Res. **2002**, *35*, 430–437

Free Energy Simulations Come of Age: Protein–Ligand Recognition

THOMAS SIMONSON,[*,†]
GEORGIOS ARCHONTIS,[‡] AND
MARTIN KARPLUS[*,§,∥]

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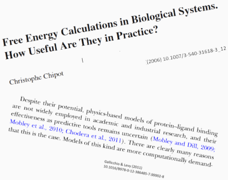


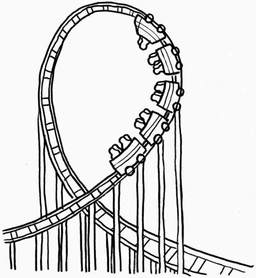
Free Energy Calculations in Biological Systems. How Useful Are They in Practice?

(2006) 10.1007/3-540-31618-3_12

Christophe Chipot

Despite their potential, physics-based models of protein–ligand binding are not widely employed in academic and industrial research, and their effectiveness as predictive tools remains uncertain (Mobley and Dill, 2009; Mobley et al., 2010; Chodera et al., 2011). There are clearly many reasons that this is the case. Models of this kind are more computationally demand-

Gallicchio & Levy (2011)
10.1016/B978-0-12-386485-7.00002-8



(c) Gwen Tomahawk

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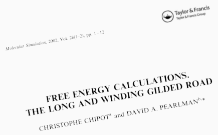

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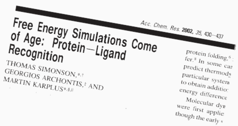

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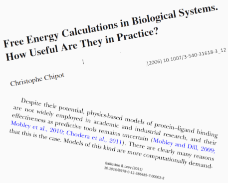

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Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field

Lingle Wang[†], Yujie Wu[†], Yuqing Deng[†], Byungchan Kim[†], Levi Pierce[†], Goran Krilov[†], Dmitry Lupyan[†], Shaughnessy Robinson[†], Markus K. Dahlgren[†], Jeremy Greenwood[†], Donna L. Romero[‡], Craig Masse[‡], Jennifer L. Knight[†], Thomas Steinbrecher[†], Thijs Beuming[†], Wolfgang Damm[†], Ed Harder[†], Woody Sherman[†], Mark Brewer[†], Ron Wester[‡], Mark Murcko[†], Leah Frye[†], Ramy Farid[†], Teng Lin[†], David L. Mobley[⊥], William L. Jorgensen[∥], Bruce J. Berne[§], Richard A. Friesner[§], and Robert Abel[*†]

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Schroedinger FEP+ (2015)

Have FES become an engineering problem?

Free Energy Methods in Drug Discovery: Who We Are, Where We Are, and Where We Are Going

Kira A. Armacost, Eric C. Gladstone*, and David C. Thompson

DOI: 10.1021/bk-2021-1397.ch011
Publication Date: November 19, 2021
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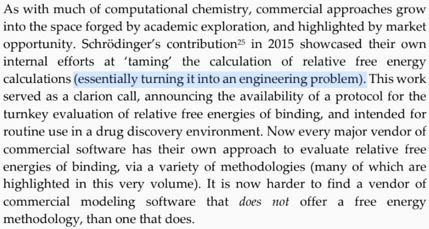
Free Energy Methods in Drug Discovery: Current State and Future Directions
Chapter 11, pp 267-287

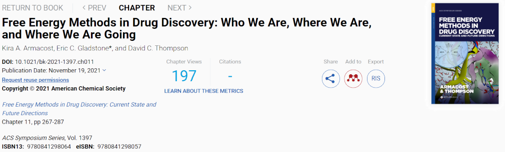
ACS Symposium Series, Vol. 1397
ISBN13: 9780841298064 **eISBN:** 9780841298057



As with much of computational chemistry, commercial approaches grow into the space forged by academic exploration, and highlighted by market opportunity. Schrödinger's contribution[25] in 2015 showcased their own internal efforts at 'taming' the calculation of relative free energy calculations (essentially turning it into an engineering problem). This work served as a clarion call, announcing the availability of a protocol for the turnkey evaluation of relative free energies of binding, and intended for routine use in a drug discovery environment. Now every major vendor of commercial software has their own approach to evaluate relative free energies of binding, via a variety of methodologies (many of which are highlighted in this very volume). It is now harder to find a vendor of commercial modeling software that *does not* offer a free energy methodology, than one that does.

(quoted from a preprint copy on researchgate.net)

Have FES become an engineering problem?



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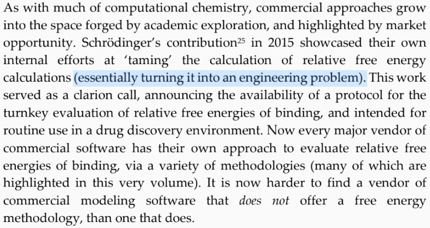

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"Although free-energy methods have made a significant impact, they aren't as robust or accurate as we'd like."
(Advertisement from OpenEye, 2024)

Grey, dear friend, is all theory.
And green the golden tree of life.
(Goethe, Faust)

Part I: What makes (has made) FES so hard?

Self-terms (aka “intraperturbed group contributions”)

Definition

$$\begin{tikzcd}
(\text{R-X})_{M1} \arrow[r, "\Delta G_{M1}^{\text{R-X}\rightarrow\text{R-Y}}"] \arrow[d, "\Delta G^{\text{R-X}}_{M1\rightarrow M2}"] & (\text{R-Y})_{M1} \arrow[d, "\Delta G^{\text{R-Y}}_{M1\rightarrow M2}"] \\
(\text{R-X})_{M2} \arrow[r, "\Delta G_{M2}^{\text{R-X}\rightarrow\text{R-Y}}"'] & (\text{R-Y})_{M2}
\end{tikzcd}$$

Definition

$\Delta\Delta G_{solv}$

M1: gas phase

M2: solution

$\Delta\Delta G_{part}$

M1: apolar solv.

M2: polar solv.

$$\begin{tikzcd}
(\text{R-X})_{M1} \arrow[r, "\Delta G_{M1}^{\text{R-X}\rightarrow\text{R-Y}}"] \arrow[d, "\Delta G_{M1\rightarrow M2}^{\text{R-X}}"] & (\text{R-Y})_{M1} \arrow[d, "\Delta G_{M1\rightarrow M2}^{\text{R-Y}}"] \\
(\text{R-X})_{M2} \arrow[r, "\Delta G_{M2}^{\text{R-X}\rightarrow\text{R-Y}}"'] & (\text{R-Y})_{M2}
\end{tikzcd}$$

$\Delta\Delta G_{bind}$

M1: solution

M2: complex

Definition

$$\begin{tikzcd}
(\text{R-X})_{M1} \arrow[r, "\Delta G_{M1}^{\text{R-X}\rightarrow\text{R-Y}}"] \arrow[d, "\Delta G^{\text{R-X}}_{M1\rightarrow M2}"] & (\text{R-Y})_{M1} \arrow[d, "\Delta G^{\text{R-Y}}_{M1\rightarrow M2}"] \\
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\end{tikzcd}$$

Self-terms[1] (“intraperturbed group contributions”)[2]: free energy contributions to $\Delta\Delta G$ resulting from energy changes in the group that is mutated (-X → -Y)

---

[1] *Biochemistry 1991, 30, 3217ff.* [2] *J. Chem. Phys. 1991, 94, 4532ff.*

Back-of-the-envelope analysis

- The potential energy function can always be unambigously separated into individual contributions
- However, there is no corresponding (unique) separation at the level of free energy differences
- Free energy contributions from changes in intramolecular terms to a free energy difference depend on the medium/environment (M1/M2). We *cannot* expect cancellation.

$\Delta\Delta G_{solv}$ of alanine vs. serine dipeptide

System / Method	$\Delta\Delta G_{solv}$ [kcal/mol]
CH_4 / CH_3OH[1]	-7.10 ± 0.63

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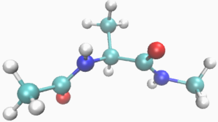
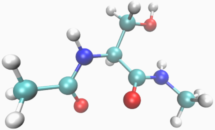
[1] Experimental data from FreeSolv.





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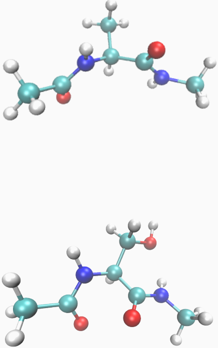
[1] Experimental data from FreeSolv. [2] Full td. cycle.



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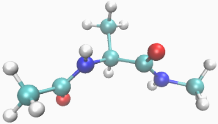
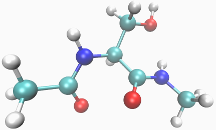
Ala / Ser[3]	-5.66 ± 0.08

[1] Experimental data from FreeSolv. [2] Full td. cycle. [3] Excluding "self-terms"





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Take-home message

- Never ignore/omit legs of a td. cycle!

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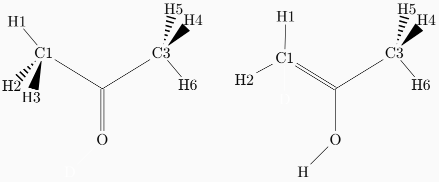
- Never ignore/omit legs of a td. cycle!
- $\Rightarrow$ But what if you have contributions that must cancel?
- $\Rightarrow$ Can one prove that a contribution is identical in both legs of the td. cycle?

⇒ Single/dual topology, dummy atoms etc.

Practical Alchemy 101

Example: acetone and its enol tautomer



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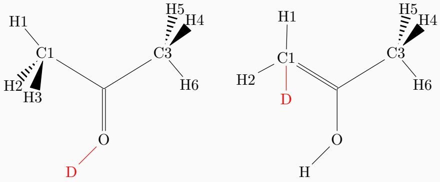

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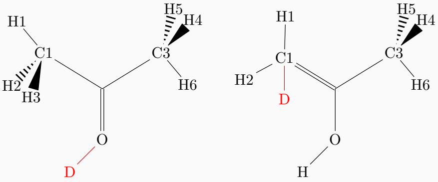
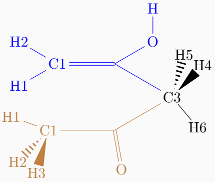


(single topology)



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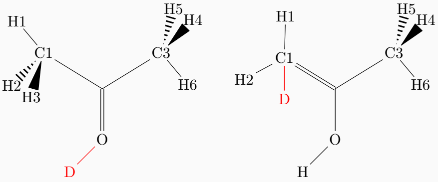

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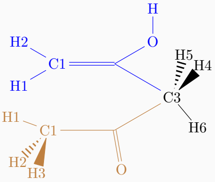

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One needs placeholders — dummy atoms $[\{\mathbf{r}_D\}]$

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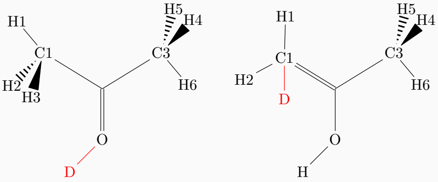

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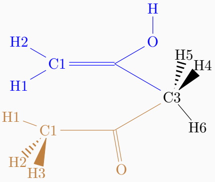

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- Should not influence the physical system [$\{\mathbf{R}\}$]

Practical Alchemy 101

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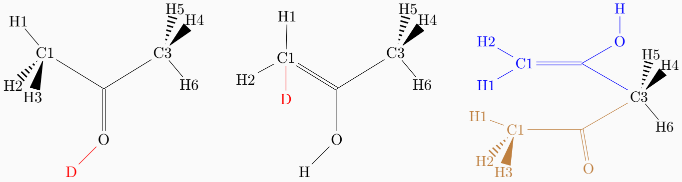
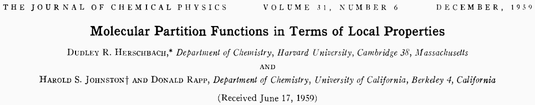


(single topology) (dual topology)

One needs placeholders — dummy atoms $[\{\mathbf{r}_D\}]$

- Should not influence the physical system $[\{\mathbf{R}\}]$
- $[\{\mathbf{r}_D\}]$ must not contribute to $\Delta\Delta G$!

My secret recipe [HJR1959]



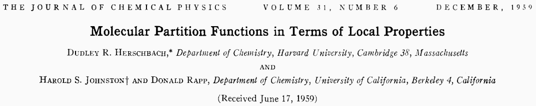
THE JOURNAL OF CHEMICAL PHYSICS VOLUME 31, NUMBER 6 DECEMBER, 1959

Molecular Partition Functions in Terms of Local Properties

Dudley R. Herschbach,* *Department of Chemistry, Harvard University, Cambridge 38, Massachusetts*
AND
Harold S. Johnston† and Donald Rapp, *Department of Chemistry, University of California, Berkeley 4, California*

(Received June 17, 1959)

My secret recipe [HJR1959]



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Think about (dummy) atoms in suitable internal coordinates

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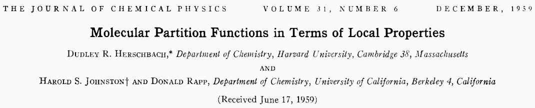

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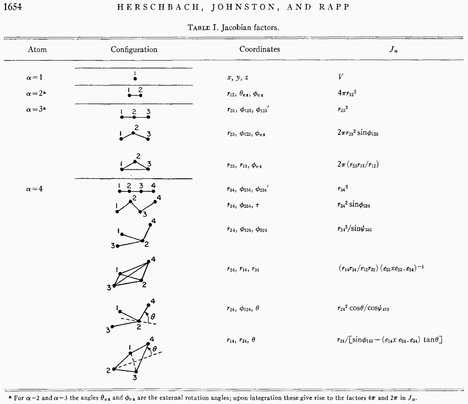

Table I. Jacobian factors.

Atom	Configuration	Coordinates	J_α
$\alpha=1$		x, y, z	V
$\alpha=2^a$		$r_{12}, \theta_{ex}, \phi_{ex}$	$4\pi r_{12}^2$
$\alpha=3^a$		$r_{23}, \phi_{123}, \phi_{123}'$	r_{23}^2
		$r_{23}, \phi_{123}, \phi_{ex}$	$2\pi r_{23}^2 \sin\phi_{123}$
		$r_{23}, r_{13}, \phi_{ex}$	$2\pi(r_{23}r_{13}/r_{12})$
$\alpha=4$		$r_{34}, \phi_{234}, \phi_{234}'$	r_{34}^2
		r_{34}, ϕ_{234}, τ	$r_{34}^2 \sin\phi_{234}$
		$r_{24}, \phi_{124}, \phi_{324}$	$r_{24}^2/\sin\psi_{341}$
		r_{24}, r_{14}, r_{34}	$(r_{14}r_{34}/r_{12}r_{32})(e_{21}xe_{23}.e_{24})^{-1}$
		$r_{24}, \phi_{124}, \theta$	$r_{24}^2 \cos\theta/\cos\psi_{413}$
		r_{14}, r_{34}, θ	$r_{24}/[\sin\phi_{143}-(e_{14}x\, e_{24}.e_{34})\tan\theta]$

[a] For $\alpha=2$ and $\alpha=3$ the angles θ_{ex} and ϕ_{ex} are the external rotation angles; upon integration these give rise to the factors 4π and 2π in J_α.

Dummy atoms 101

- Separability: $Z \overset{?}{=} Z(\{\mathbf{R}\}) \times Z(b(\{\mathbf{r}_D\}))$

Dummy atoms 101

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- Focus on "junction"

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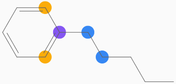

Dummy atoms 101

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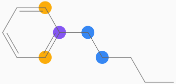
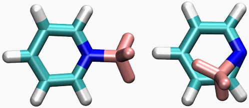


- Avoid needless sampling



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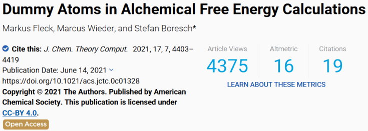

Dummy Atoms in Alchemical Free Energy Calculations

Markus Fleck, Marcus Wieder, and Stefan Boresch*

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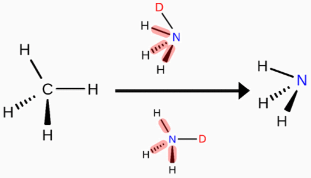

Dummy atoms 101

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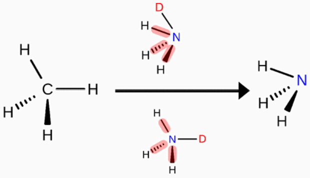

“Why was this paper still necessary in 2021?”

Applying [HJR1959]: Restraints in absolute binding free energy calculations

ABFE calculations (double decoupling method)

Need for restraints[1]

$$\Delta A_{rest} = -k_B T \ln \frac{Z_{rest}}{8\pi^2 V_0}$$

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[1] *Biophys. J. 1997, 72, 1047ff.*

ABFE calculations (double decoupling method)

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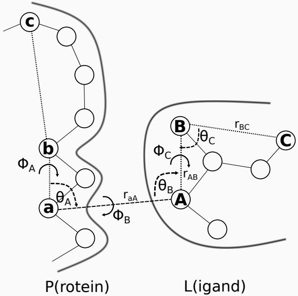

$$\Delta A_{rest} = -k_B T \ln \frac{Z_{rest}}{8\pi^2 V_0}$$

Using [HJR1959][2]: $Z_{rest} \approx$

$$r^2_{a,A,0} \sin\theta_{A,0} \sin\theta_{B,0} \frac{(2\pi k_B T)^3}{(K'_r K'_{\theta_A} K'_{\theta_B} K'_{\phi_A} K'_{\phi_B} K'_{\phi_C})^{1/2}}$$

---

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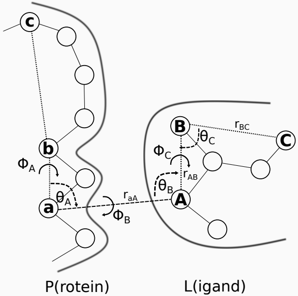

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The much more complicated expressions by Schroedinger are not necessary[3]

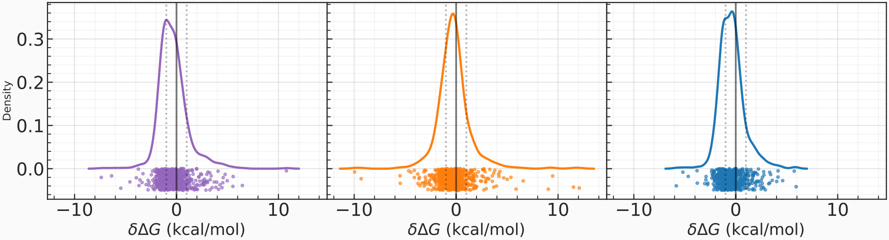
[1] *Biophys. J. 1997, 72, 1047ff.* [2] *J. Phys. Chem. B 2003, 107, 9535ff.* [3] *ChemRxiv: 10.26434/chemrxiv-2023-8s9dz-v3, subm. JCIM*

Part II: Where do we stand — ongoing challenges

A simple(?) task

Solvation free energies (FreeSolv)

AMBER/GAFF | CGenFF | OpenFF 2.0



JCAMD 2014, 28, 711

JCTC 2023, 19, 5988

10.26434/chemrxiv-2023-8jgjq
subm. JPC B

$$\delta\Delta G = \Delta G^{exp}_{solv} - \Delta G^{calc}_{solv}$$

Beyond additive force fields → QM/MM, ML/MM (ANI-2x/MM[1])

$$\begin{tikzcd}
X^{MM}_{gas} \arrow[r, "\Delta G^{\mathrm{gas}}_{\mathrm{MM}\rightarrow\mathrm{ANI\text{-}2x}}"] \arrow[d, "\Delta G^{MM}_{solv}"] & X^{\mathrm{ANI\text{-}2x}}_{gas} \arrow[d, dashed, "\Delta G^{\mathrm{ANI\text{-}2x/MM}}_{solv}"] \\
(X)^{MM}_{aqu} \arrow[r, "\Delta G^{\mathrm{aqu}}_{\mathrm{MM}\rightarrow\mathrm{ANI\text{-}2x/MM}}"'] & (X)^{\mathrm{ANI\text{-}2x/MM}}_{aqu}
\end{tikzcd}$$

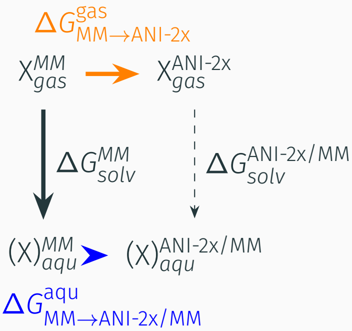
- indirect cycle
- OpenMM-ML[2]
- non-equil. switches[3]

---

[1] *10.26434/chemrxiv-2023-8jgjq* [2] *github.com/openmm/openmm-ml*

[3] *github.com/wiederm/endstate_correction*

Beyond additive force fields → QM/MM, ML/MM (ANI-2x/MM[1])



$\Delta G^{gas}_{MM \rightarrow ANI\text{-}2x}$

$X^{MM}_{gas} \rightarrow X^{ANI\text{-}2x}_{gas}$

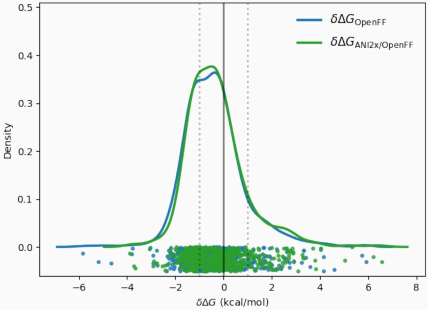
ΔG^{MM}_{solv}

$\Delta G^{ANI\text{-}2x/MM}_{solv}$

$(X)^{MM}_{aqu} \rightarrow (X)^{ANI\text{-}2x/MM}_{aqu}$

$\Delta G^{aqu}_{MM \rightarrow ANI\text{-}2x/MM}$

- indirect cycle
- OpenMM-ML[2]
- non-equil. switches[3]



- no real improvement

---

[1] 10.26434/chemrxiv-2023-8jgjq [2] github.com/openmm/openmm-ml
[3] github.com/wiederm/endstate_correction

Beyond additive force fields → QM/MM, ML/MM (ANI-2x/MM)

Should I have been surprised?

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- Mechanical embedding

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What next?

- Better (=electrostatic) embedding

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- Indirect → *direct* FES?

Beyond additive force fields → QM/MM, ML/MM (ANI-2x/MM)

Should I have been surprised?

- Mechanical embedding

What next?

- Better (=electrostatic) embedding
- Indirect → *direct* FES?
  ⇒ Dummy atoms and end point issues in QM or ML?

Aren’t we rushing ahead of ourselves?

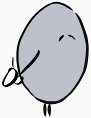
An observation

Results agree with experiment

(c) S. Tkaczyk

An observation

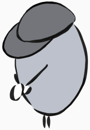
Results do not agree with experiment



(c) S. Tkaczyk

An observation

Results do not agree with experiment



(c) S. Tkaczyk

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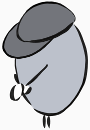


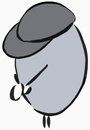
(c) S. Tkaczyk

The “usual suspects”

- Description of interactions
- Sampling
- System setup / technical issues (change in net charge)
- Errors in carrying out transformations

An observation

Results do not agree with experiment


(c) S. Tkaczyk

The "usual suspects"

- Description of interactions
- Sampling
- System setup / technical issues (change in net charge)
- Errors in carrying out transformations

Question(s): Can we distinguish between these??

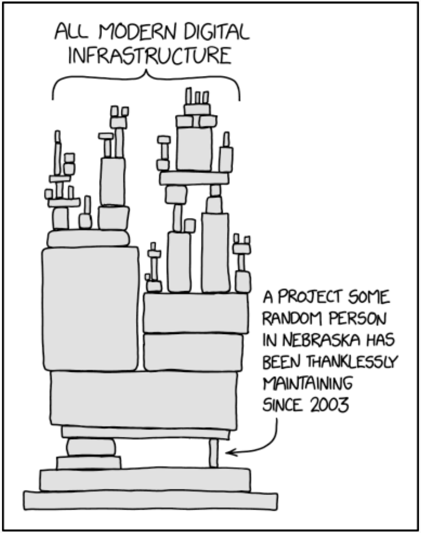
Are we at least trying?

Challenge

When carrying out FES with today's "usual protocols:"

- flexibility of the free ligand
- flexibility of the binding site / the bound ligand
- bias from the starting pose

The state of FES?



(c) R. Munroe (https://imgs.xkcd.com/comics/dependency.png)

Thank you, etc.

Acknowledgements

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- Martin Karplus

- Martin Leitgeb, Franz Tettinger
- Gerhard König
- Markus Fleck
- Marcus Wieder, Johannes Karwounopoulos, Sara Tkaczyk, Anna Picha
- Andreas Schöller

Funding & Support

- University of Vienna
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Collaboration

- USF (Lee Woodcock, Fiona Kearns, Phillip Hudson)
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TUE EV. POSTER

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Last, but not least

My thanks and best wishes to the Titans, Trendsetters, and Talispersons!

Thank you!
Questions?

Self terms: a closer look

Consider a ligand/solute L in some medium M (gas phase, aqueous solution, complexed to a receptor, ...)

The ligand L = R – X consists of scaffold R and a functional group X. Thus, we have:

$$\{\mathrm{r}\} = \{\mathrm{r}_\mathrm{L}, \mathrm{r}_\mathrm{M}\} \qquad \{\mathrm{r}_\mathrm{L}\} = \{\mathrm{r}_\mathrm{R}, \mathrm{r}_\mathrm{X}\}$$

$$\begin{aligned} U(\{\mathrm{r}\}) &= U_L(\{\mathrm{r}_\mathrm{L}\}) + U_{L\text{-}M}(\{\mathrm{r}_\mathrm{L}, \mathrm{r}_\mathrm{M}\}) + U_M(\{\mathrm{r}_\mathrm{M}\}) = \\ &= \underbrace{U_{R\cdot R}(\{\mathrm{r}_\mathrm{R}\}) + U_{R\cdot X}(\{\mathrm{r}_\mathrm{R}, \mathrm{r}_\mathrm{X}\}) + U_{X\cdot X}(\{\mathrm{r}_\mathrm{X}\})}_{U_L} + \\ &+ \underbrace{U_{R\text{-}M}(\{\mathrm{r}_\mathrm{R}, \mathrm{r}_\mathrm{M}\}) + U_{X\text{-}M}(\{\mathrm{r}_\mathrm{X}, \mathrm{r}_\mathrm{M}\})}_{U_{L\text{-}M}} + U_M(\{\mathrm{r}_\mathrm{M}\}) \end{aligned}$$

Self terms: a closer look

Let's now consider the mutation

$$\overbrace{(\mathrm{R}{-}\mathrm{X}_0)}^{\mathrm{L}_0}{}_{\mathrm{M}} \xrightarrow{\Delta G_{\mathrm{M}}^{\mathrm{L0}\rightarrow\mathrm{L1}}} \overbrace{(\mathrm{R}{-}\mathrm{X}_1)}^{\mathrm{L}_1}{}_{\mathrm{M}}$$

$$\lambda = 0 \colon \mathrm{R}{-}\mathrm{X}_0 \qquad \lambda = 1 \colon \mathrm{R}{-}\mathrm{X}_1$$

and make the potential energy dependent on λ:

$$\begin{aligned} U(\{\mathbf{r}\},\lambda) &= \underbrace{U_{R\text{-}R}(\{\mathbf{r}_\mathrm{R}\}) + U_{R\text{-}X}(\{\mathbf{r}_\mathrm{R},\mathbf{r}_\mathrm{X}\},\lambda) + U_{X\text{-}X}(\{\mathbf{r}_\mathrm{X}\},\lambda)}_{U_L} + \\ &+ \underbrace{U_{R\text{-}M}(\{\mathbf{r}_\mathrm{R},\mathbf{r}_\mathrm{M}\}) + U_{X\text{-}M}(\{\mathbf{r}_\mathrm{X},\mathbf{r}_\mathrm{M}\},\lambda)}_{U_{L\text{-}M}} + U_M(\{\mathbf{r}_\mathrm{M}\}) \end{aligned}$$

TI: $$\Delta A = A(\lambda=1) - A(\lambda=0) = \int_0^1 \left(\frac{dA}{d\lambda}\right) d\lambda = \int_0^1 \left\langle \frac{\partial U(\{\mathbf{r}\},\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda$$

Self terms: a closer look

$$\left\langle \frac{\partial U(\{\mathrm{r}\}, \lambda)}{\partial \lambda} \right\rangle_\lambda =$$

$$\underbrace{\left\langle \frac{\partial U_{R\text{-}X}(\{\mathrm{r_R}, \mathrm{r_X}\}, \lambda)}{\partial \lambda} \right\rangle_\lambda + \left\langle \frac{\partial U_{X\text{-}X}(\{\mathrm{r_X}, \mathrm{r_X}\}, \lambda)}{\partial \lambda} \right\rangle_\lambda}_{\Rightarrow\text{self-term(s)}} + \left\langle \frac{\partial U_{X\text{-}M}(\{\mathrm{r_X}, \mathrm{r_M}\}, \lambda)}{\partial \lambda} \right\rangle_\lambda$$

But:

$$\left\langle \frac{\partial U_{R\text{-}X}(\{\mathrm{r_R}, \mathrm{r_X}\}, \lambda)}{\partial \lambda} \right\rangle_\lambda = \frac{1}{Z(\lambda)} \int d\{\mathrm{r_R}\} d\{\mathrm{r_X}\} d\{\mathrm{r_M}\} \frac{\partial U_{R\text{-}X}(\{\mathrm{r_R}, \mathrm{r_X}\}, \lambda)}{\partial \lambda} \times$$
$$\exp(-\beta U(\{\mathrm{r_R}, \mathrm{r_X}, \mathrm{r_M}\}, \lambda))$$

etc. The integral cannot be separated further. The “self-term” contributions depend on the medium/environment M.