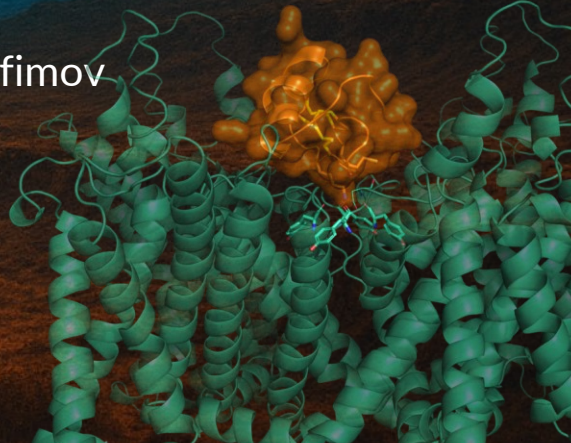
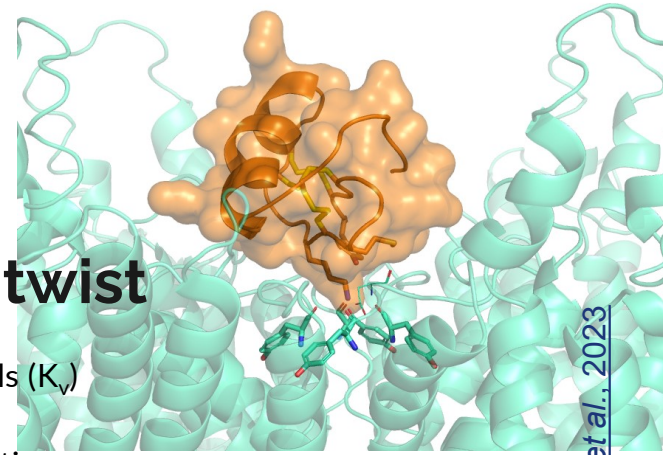
A long, hard road to physically correct calculation of protein–protein binding free energies

A. Chugunov, V. Tabakmakher, I. Panina, A. Vassilevski, Yu. Trofimov



BCADD — October 22nd, 2025

ChTx and ChTx^{M29I}: the long-awaited twist



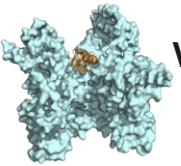
- Charybdotoxin (ChTx, α-KTx1.1) is a classic blocker of potassium channels (K_v) from a scorpion *Leiurus hebraeus* venom
- ChTx — high-affinity selective K_V1.3 ligand, although a single M29I mutation dramatically switches selectivity K_V1.3 → K_V1.2

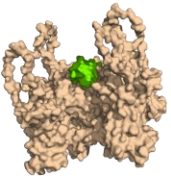
Toxin	Sequence	IC50 values, nM				Ref.
		Kv1.1	Kv1.2	Kv1.3	Kv1.6	
ChTx	ZFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS	1500	9	0,19	22	Garcia et al., 1994; Takacs et al., 2009
ChTx [M29I]	ZFTNVSCTTSKECWSVCQRLHNTSRGKCINKKCRCYS	2000 (3.1 %)	0,006	4,1	2000 (9.6 %)	Gigolaev et al., 2022

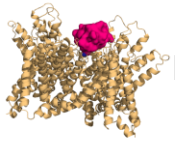
- This selectivity switch is likely of evolutionary origin:
  - most α-KTx contain a KC[M/I]N motif
  - M/I importance is proven by a mutagenesis
  - M↔I switch is caused by mutation of just third nucleotide in the codon

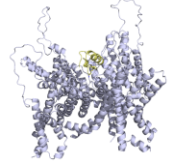
Gigolaev *et al.*, 2022 — Kuzmenkov *et al.*, 2023

“Vanilla” Molecular Dynamics (MD): 🤦









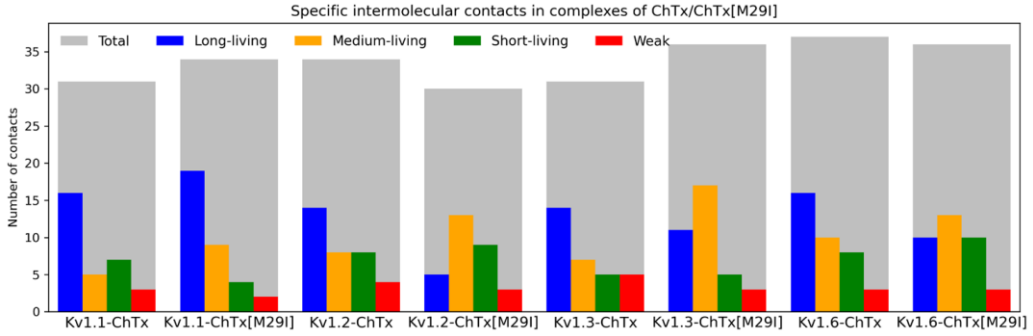
What was done:

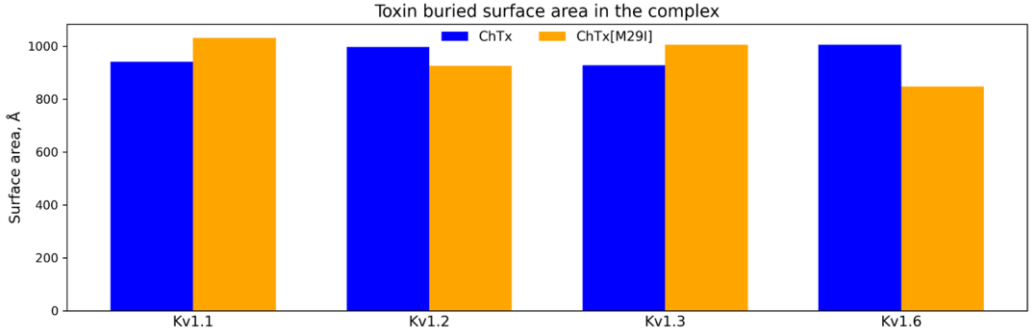
- Models of complexes of ChTx and ChTx^{M29I} with $K_V1–3$ channels
- MD in a lipid membrane (500 ns)
- Intermolecular contacts analysis
- Calculation of solvent-accessible surfaces

Results: No agreement with experiments :-(

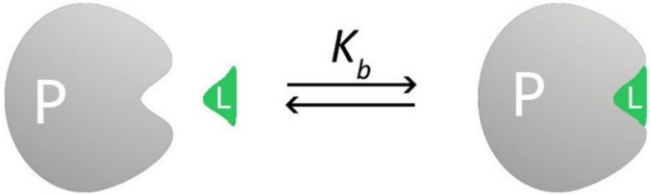
❌ Low contacts number in high-affinity complexes, and *vice versa*

❌ Low interaction areas in high-affinity complexes (or do not differ)



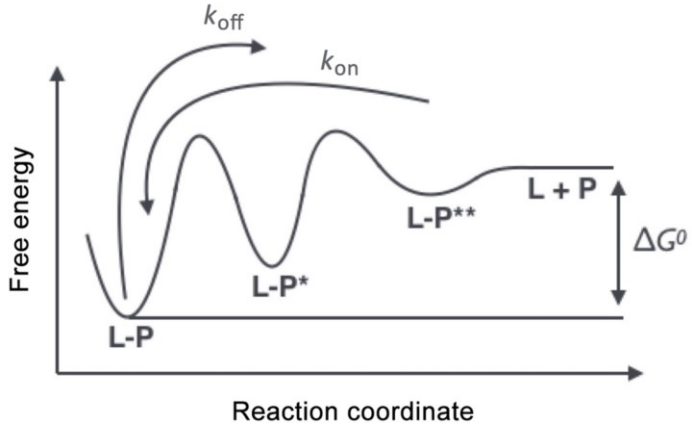


The problem of calculation of binding free energies



$$P + L \leftrightarrow PL$$

$$K_d = \frac{[P] \cdot [L]}{[PL]} = \frac{1}{K_b}$$



$$\Delta G^\circ = -RT \cdot \ln\left(K_b \cdot C^\circ\right) = RT \cdot \ln\left(\frac{K_d}{C^\circ}\right)$$

(where C° — standard concentration 1 M)

Limongelli 2020

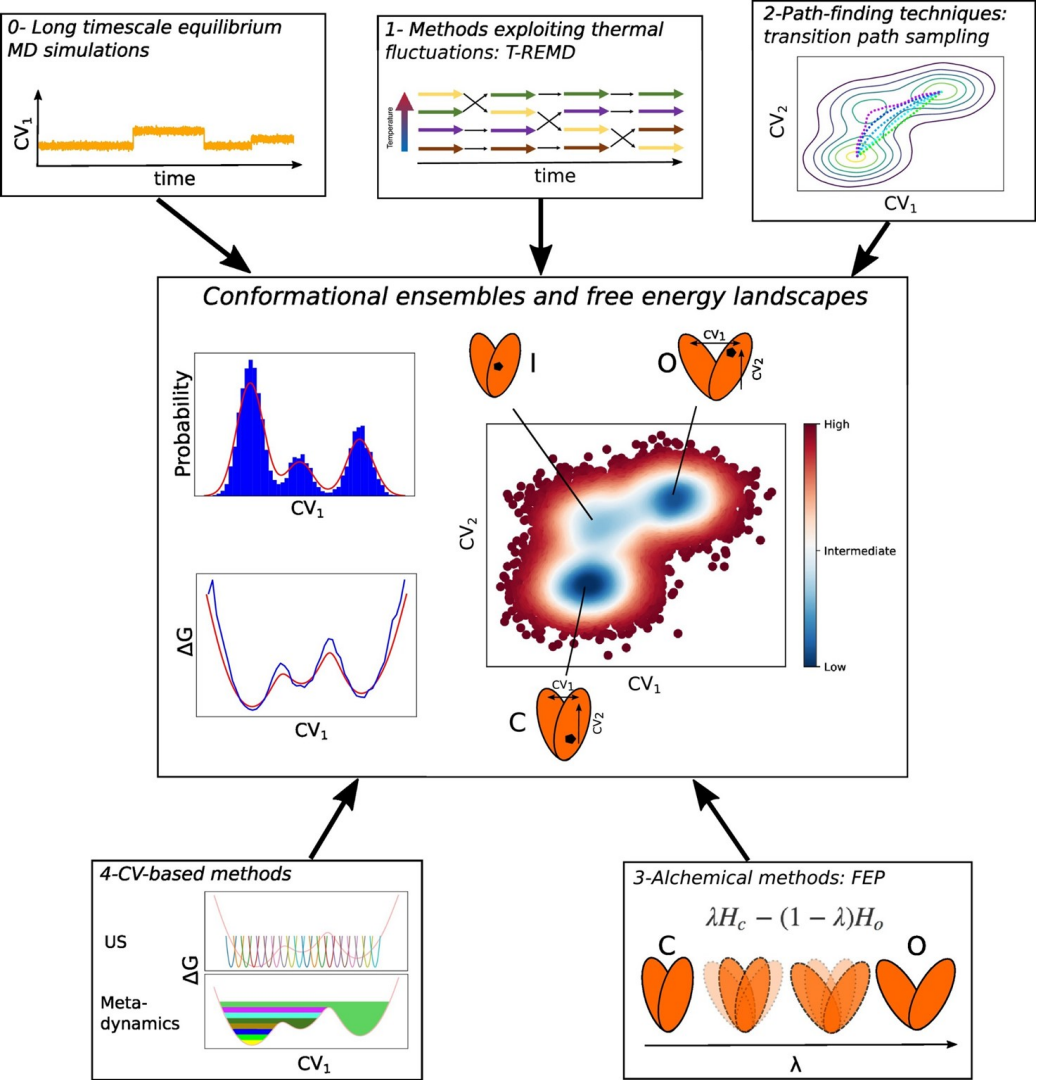
Free energy is a function of probabilities and paths…

Yu. Trofimov, personal communication

Ca^{2+} – $H_3C\text{-}COO^-$

- …which could be calculated from MD simulations…
- …but it's better to predefine path (collective variable)

Physics world



Problems of Molecular Dynamics

- Physical inaccuracy (force fields)
- Lack of sampling and computing power
- Complicated analysis

Existing approaches:

- "Vanilla" MD
- Replica exchange
- Modeling of transition paths
- "Collective variable" (CV) methods:
  - Umbrella sampling
  - Metadynamics
  - Adaptive weight histogram
- Alchemical transformations

Harpole & Delemotte, 2018

British chemist, Honorary Professor of Computer Science, Director of the Centre for Computational Science and Associate Director of the Advanced Research Computing Centre at University College London.

Peter Coveney p.v.coveney@ucl.ac.uk через outlook.com 25 авг. 2024 г., 13:25

кому: мне, Peter

Dear Dr Chugunov,

There are some studies published on protein-protein interaction energies. You can find some examples in research and review papers, DOI:10.3389/fmolb.2017.00087 and DOI:10.1002/wcms.1448, for example. It should be noted that the binding energies for such protein-protein systems typically have large uncertainties. Our studies of peptide-MHC systems showed a difference of ~40 kcal/mol in the binding free energies from simulations differing only in their initial velocities. For small molecule binding to proteins, we recommend an ensemble based approach with 25 replicas and 4 ns production runs for the molecular systems with well defined initial structures. When the initial structures are less reliable, longer simulations and/or more replicas will be needed. For protein-protein interaction, the interaction energies between the two proteins from MMPBSA-based approaches will be larger than these in peptide-MHC systems we have studied. Such studies will need much longer simulation times.

If you want to study the binding affinity changes upon mutations in protein–protein interactions, alchemical approaches will be more suitable than the end-point methods as the uncertainties are largely cancelled out in the former approaches. We have done a few such studies some years ago, on peptide-MHCs binding with same TCR; good agreement was obtained between the calculations and the experimental data.

Best regards,

Peter

ach

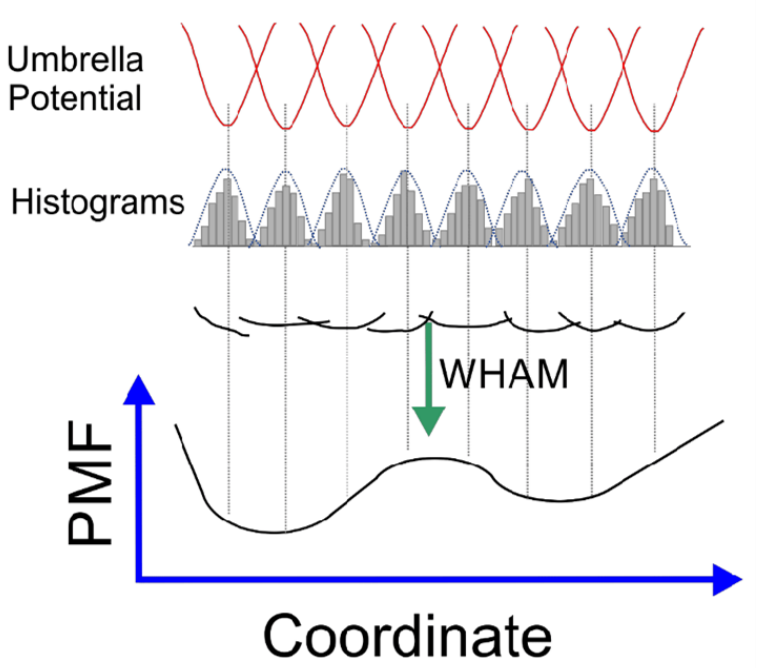
although as far as I

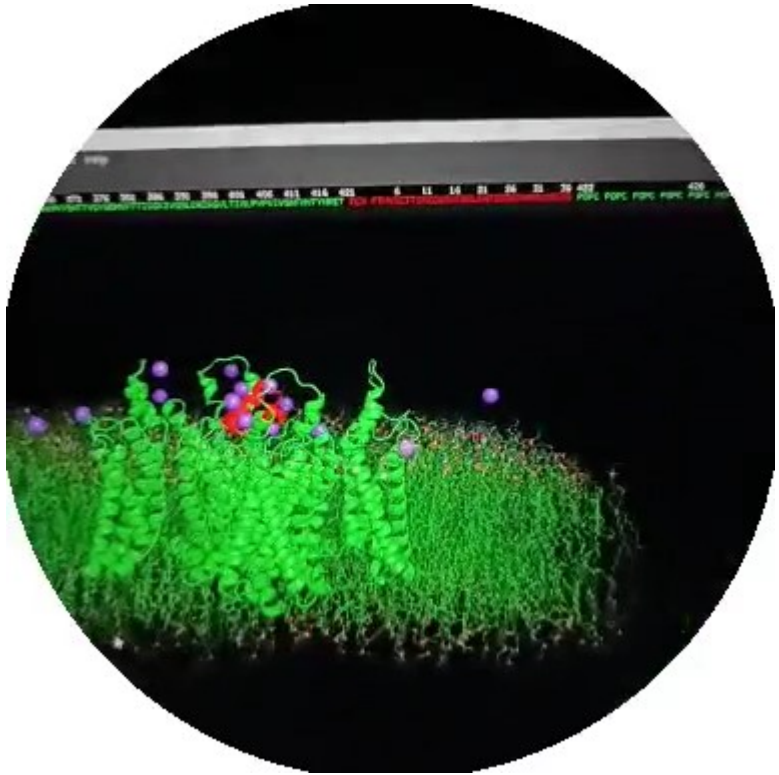
l binding sites. However,

channels and their pore

naybe you authored a paper

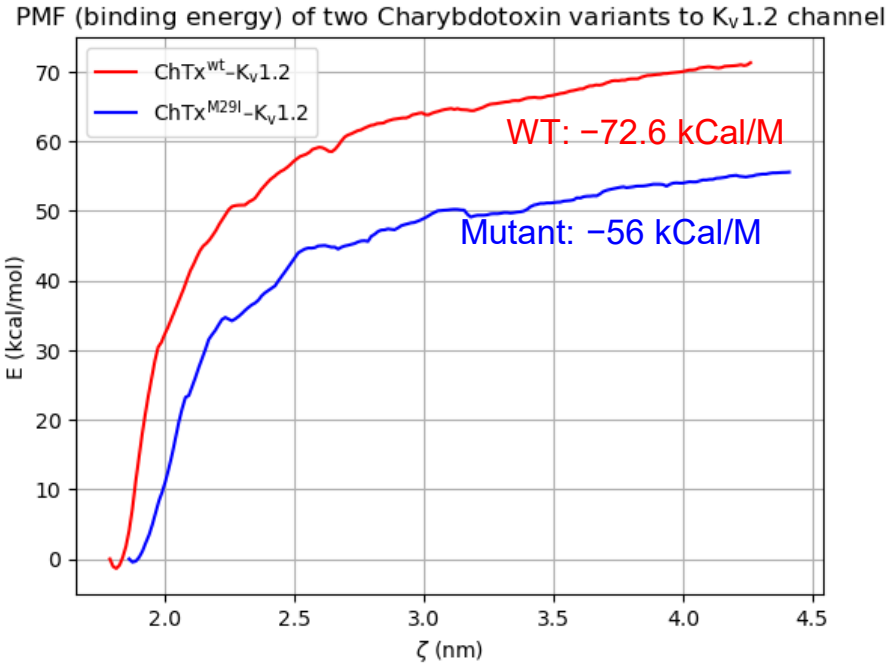
Umbrella Sampling (US-MD)



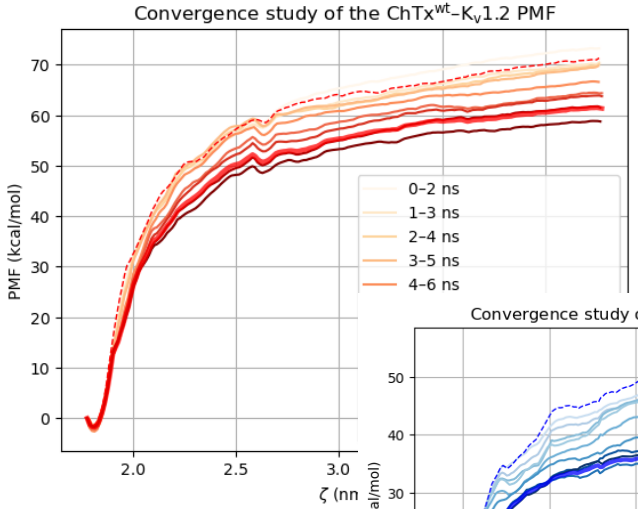


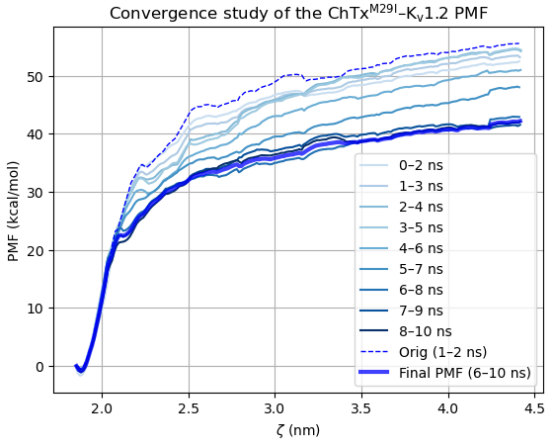
Rög *et al.*, 2021

US-MD: epic fail 🤦

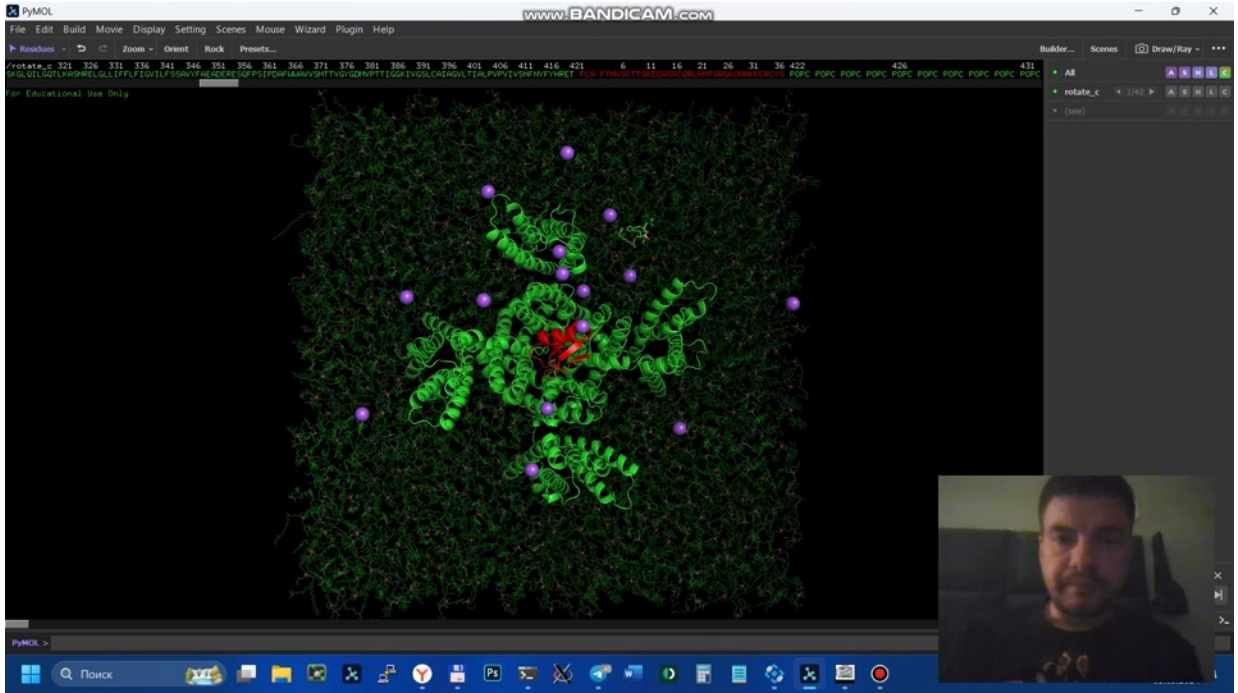


Convergence study did not help





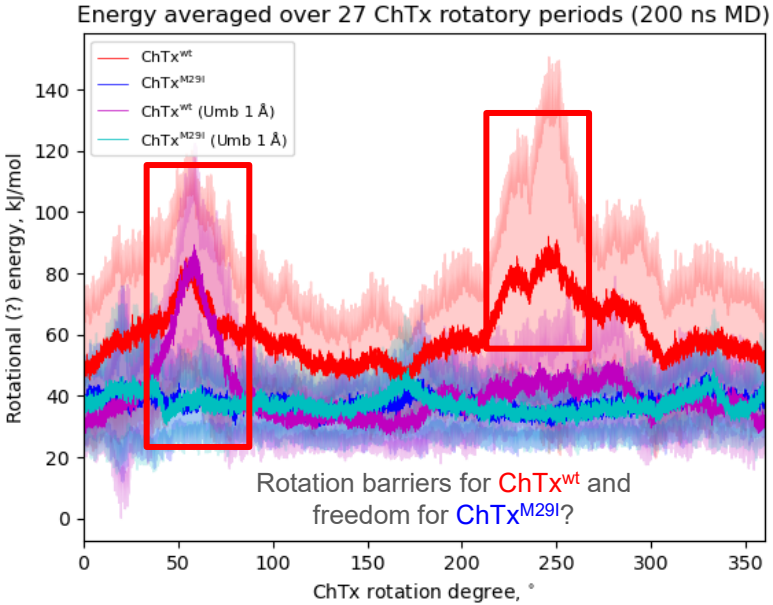
Forced rotation MD: 🤷

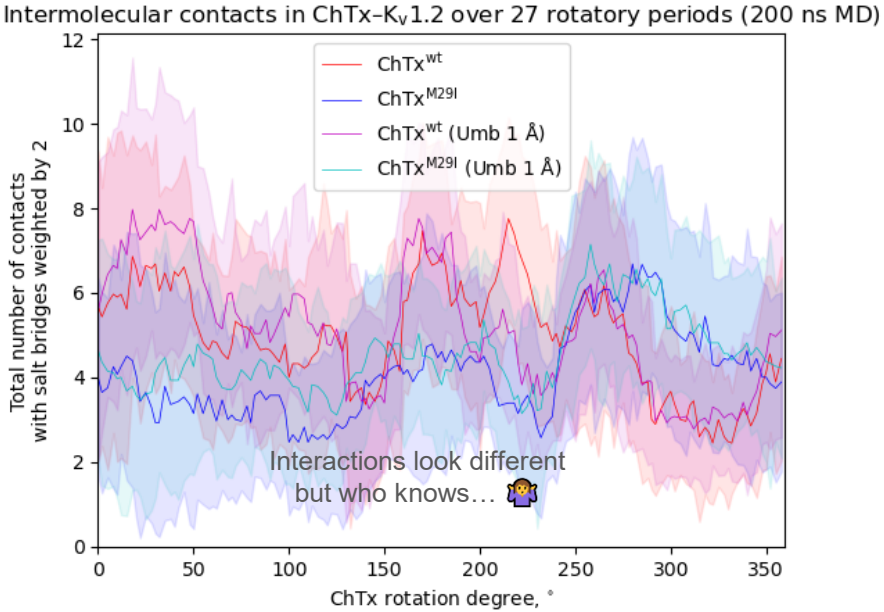


GROMACS allows for constant speed rotation:

- Torque
- “Energy”
- ❌ No true ΔG calculation

Forced rotation MD: first hints on alternative modes of binding





Advanced Weight Histogram: do all paths lead to Rome?

JCTC Journal of Chemical Theory and Computation

pubs.acs.org/JCTC Article

Do All Paths Lead to Rome? How Reliable is Umbrella Sampling Along a Single Path?

Noora Aho,* Gerrit Groenhof,* and Pavel Buslaev*

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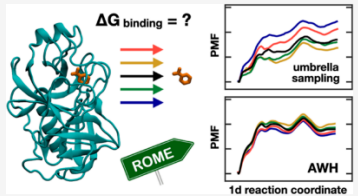
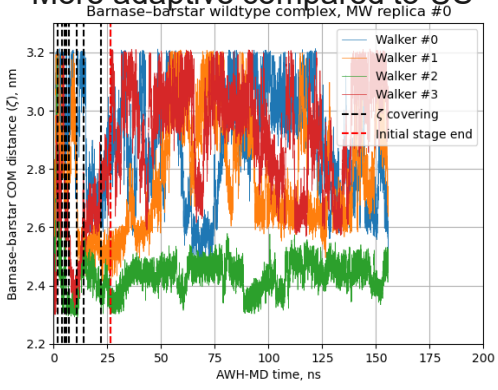
ABSTRACT: Molecular dynamics (MD) simulations are widely applied to estimate absolute binding free energies of protein−ligand and protein−protein complexes. A routinely used method for binding free energy calculations with MD is umbrella sampling (US), which calculates the potential of mean force (PMF) along a single reaction coordinate. Surprisingly, in spite of its widespread use, few validation studies have focused on the convergence of the free energy computed along a single path for specific cases, not addressing the reproducibility of such calculations in general. In this work, we therefore investigate the reproducibility and convergence of US along a standard distance-based reaction coordinate for various protein−protein and protein−ligand complexes, following commonly used guidelines for the setup. We show that repeating the complete US workflow can lead to differences of 2−20 kcal/mol in computed binding free energies. We attribute those discrepancies to small differences in the binding pathways. While these differences are unavoidable in the established US protocol, the popularity of the latter could hint at a lack of awareness of such reproducibility problems. To test if the convergence of PMF profiles can be improved if multiple pathways are sampled simultaneously, we performed additional simulations with an adaptive-biasing method, here the accelerated weight histogram (AWH) approach. Indeed, the PMFs obtained from AHW simulations are consistent and reproducible for the systems tested. To the best of our knowledge, our work is the first to attempt a systematic assessment of the pitfalls in one the most widely used protocols for computing binding affinities. We anticipate therefore that our results will provide an incentive for a critical reassessment of the validity of PMFs computed with US, and make a strong case to further benchmark the performance of adaptive-biasing methods for computing binding affinities.

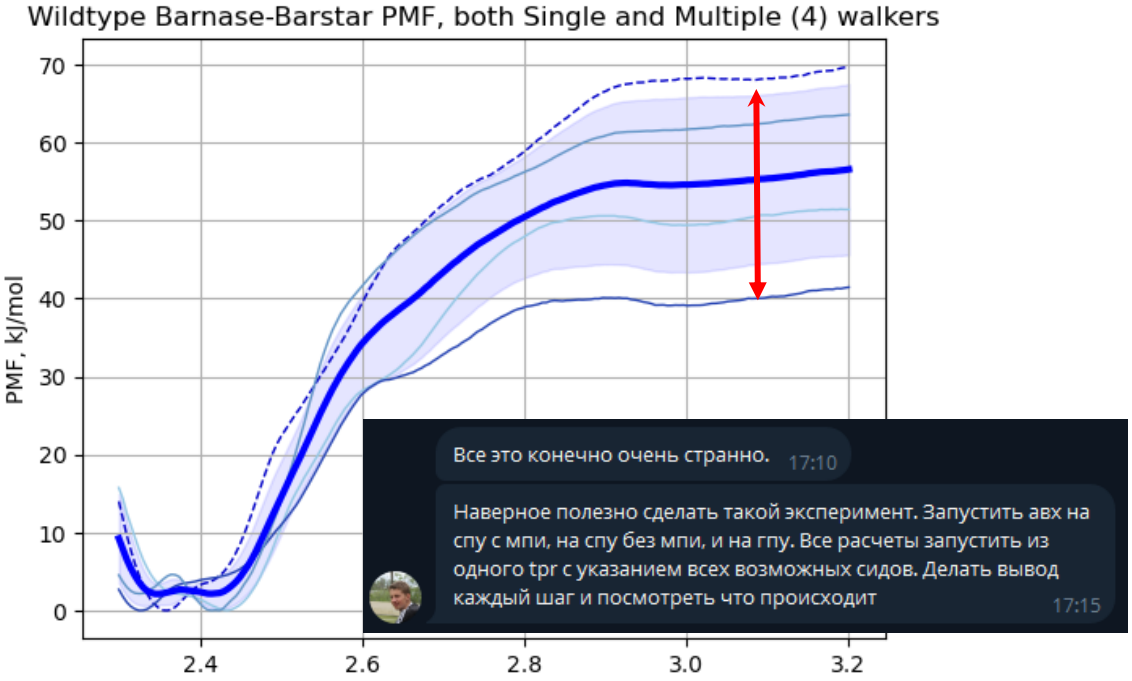


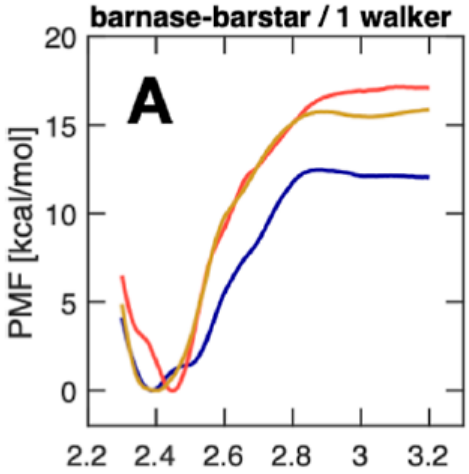
AWH is one of enhanced sampling methods

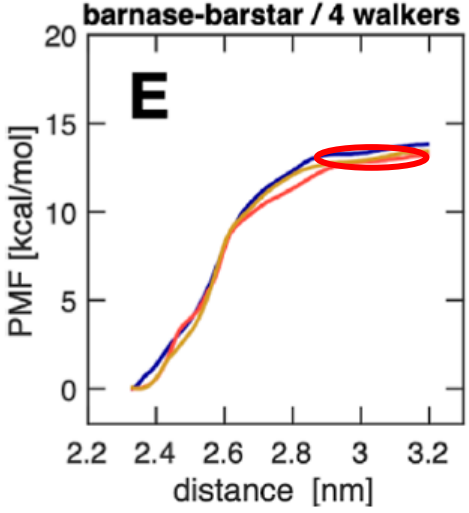
- Do not require for manual “windows” selection
- Supports “multiwalkers”
- Dynamic update of biasing function
- Two-stage biasing
- More adaptive compared to US



AWH: where is reproducibility? 😮







AWH: barnase/barstar mutations

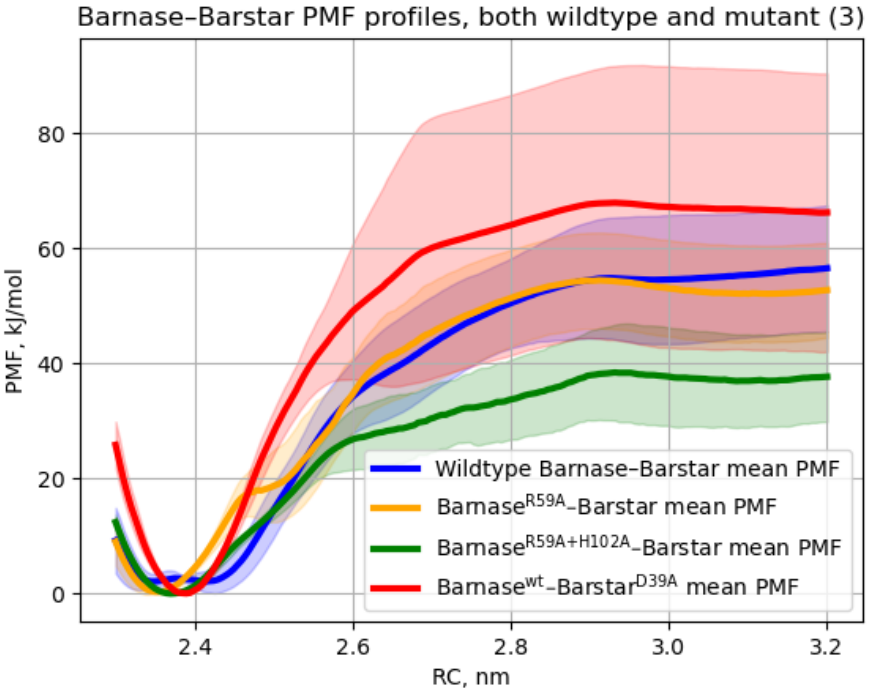


Table V: Dissociation Coefficients of Barnase–Barstar Complexes[a]

barnase	barstar	K_d (M)	ΔG (kcal/mol)	$\Delta\Delta G$ (kcal/mol)
wild-type	wild-type	6×10^{-14}	18.0	0.0
wild-type	C40,82A	2×10^{-13}	17.3	–0.7
R59K	wild-type	4×10^{-12}	15.6	–2.4
R59K	C40,82A	2.5×10^{-11} [b]	14.3	–3.7
R59A	wild-type	1.5×10^{-10} [b]	13.3	–4.7
R59A	C40,82A	1.7×10^{-0} [b]	12.0	–6.0
H102Q	wild-type	1.3×10^{-10}	13.5	–4.5
H102Q	C40,82A	1.7×10^{-9}	12.0	–6.0
H102D	wild-type	1.3×10^{-10}	13.5	–4.5
H102G	wild-type	6×10^{-9}	11.2	–6.8
H102A	wild-type	7×10^{-9}	11.1	–6.9
H102L	wild-type	2.5×10^{-8}	10.4	–7.6
H102Q,R59K	wild-type	7×10^{-9}	11.1	–6.9
H102Q,R59A	wild-type	4×10^{-7}	8.7	–9.3

assumption that K_d
btained directly by

есть много вопросов на которые у меня нет ответов, и пока этих ответов у меня нет, я не знаю как правильно нужно было бы моделировать такой комплекс. Если структурные изменения минимальны, то я бы моделировал с помощью алхимии, потому что она будет быстрее и точнее всего остального. Если моделировать PMF, то нужно очень хорошо разобраться с тем какие взаимодействия бывают на PPI и насколько хорошо современные открытые силовые поля их описывают (charmm и amber скорее всего не очень хороши для большинства взаимодействий). Дальше я бы долго моделировал белки отдельно и комплексы, без вытягивания - типа несколько микросекунд как минимум и смотрел бы - какие изменения мы видим - является ли начальная структура устойчивой или нет. Я не знаю, что из этого, ты уже сделал/хотел бы сделать. Поэтому однозначно сказать, почему что-то не сходится с эксперементом - я не могу. Глобально, я думаю, что любое большое изменение - мы предсказываем количественно достаточно плохо

16:48

В общем мое мнение - это до сих пор не является простой задачей, и было бы неплохо если бы у людей это мнение было нормой

16:55

Hartley, 1993

Alchemical transformations

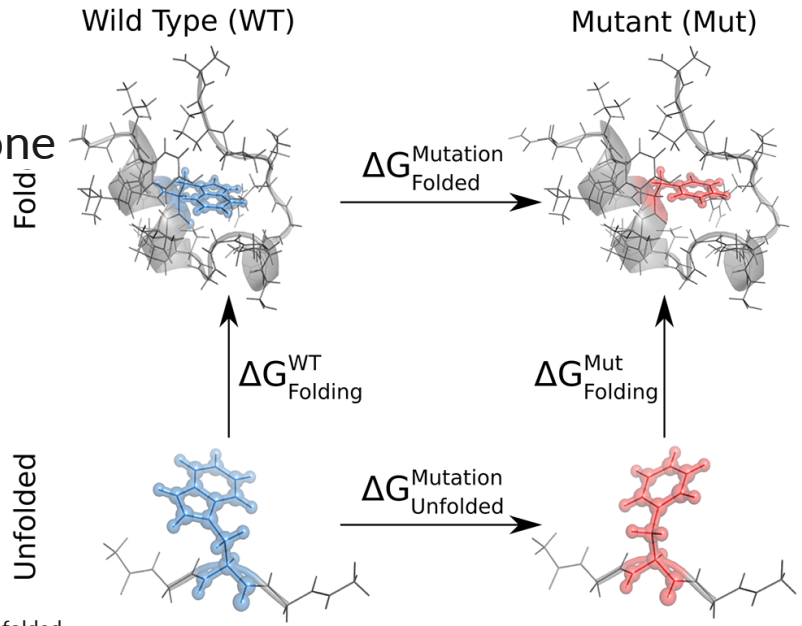
Molecular alchemy allows to transform one molecule (part) into another in MD or “annihilate” them

- At the same time, energy is measured
- Transformation is guided by λ: 0→1

$\Delta\Delta G^{Mutation}_{Folding}$ could be calculated:

1. by folding modelind (extensive)
2. More easily: comparing $\Delta G^{Mutation}_{Folded}$ and $\Delta G^{Mutation}_{Unfolded}$

Thermodynamics and statphysics say: this is the same!



$$\Delta\Delta G^{Mutation}_{Folding} = \underbrace{\Delta G^{Mut}_{Folding} - \Delta G^{WT}_{Folding}}_{1} = \underbrace{\Delta G^{Mutation}_{Folded} - \Delta G^{Mutation}_{Unfolded}}_{2}$$

Gapsys et al., 2017

Techniques of non-equilibrium alchemy

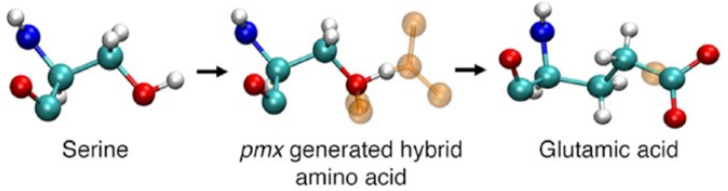
The work done is no less than the free energy change between the initial and final states.

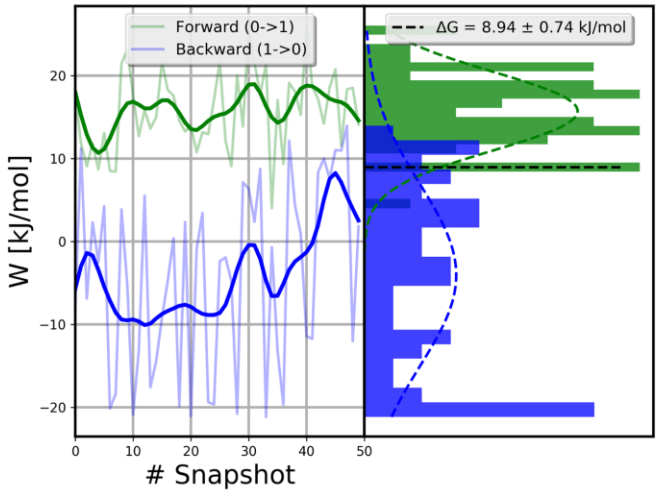
$$\langle W(\tau) \rangle \geq \Delta G$$

This work could be computed as the transformation goes forward ($\lambda_{0\to1}$)
and backward ($\lambda_{1\to0}$)

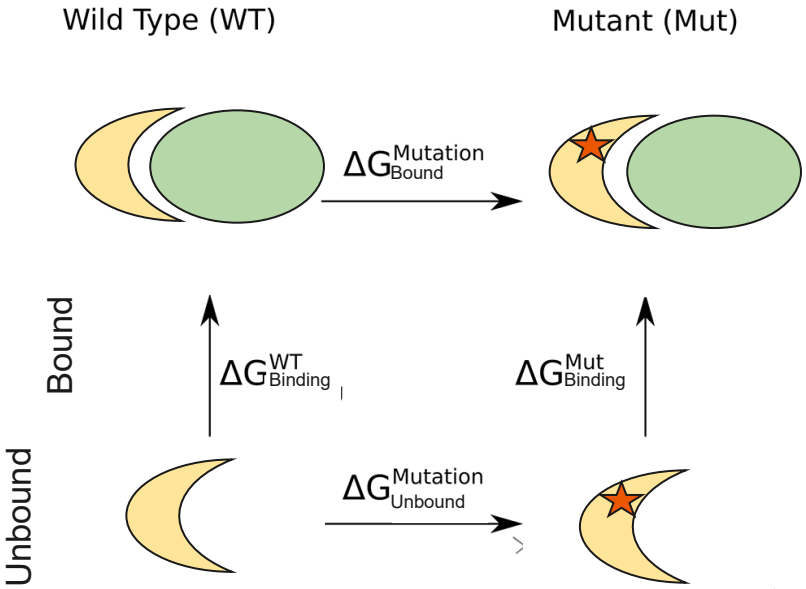
$$W(\tau) = \int_{\lambda=0}^{\lambda=1} \frac{\partial H(\vec{x}, \vec{v}, \lambda)}{\partial \lambda} d\lambda$$

H — system Hamiltonian, x — phase coordinates, v — velocities,
λ — transformation parameter

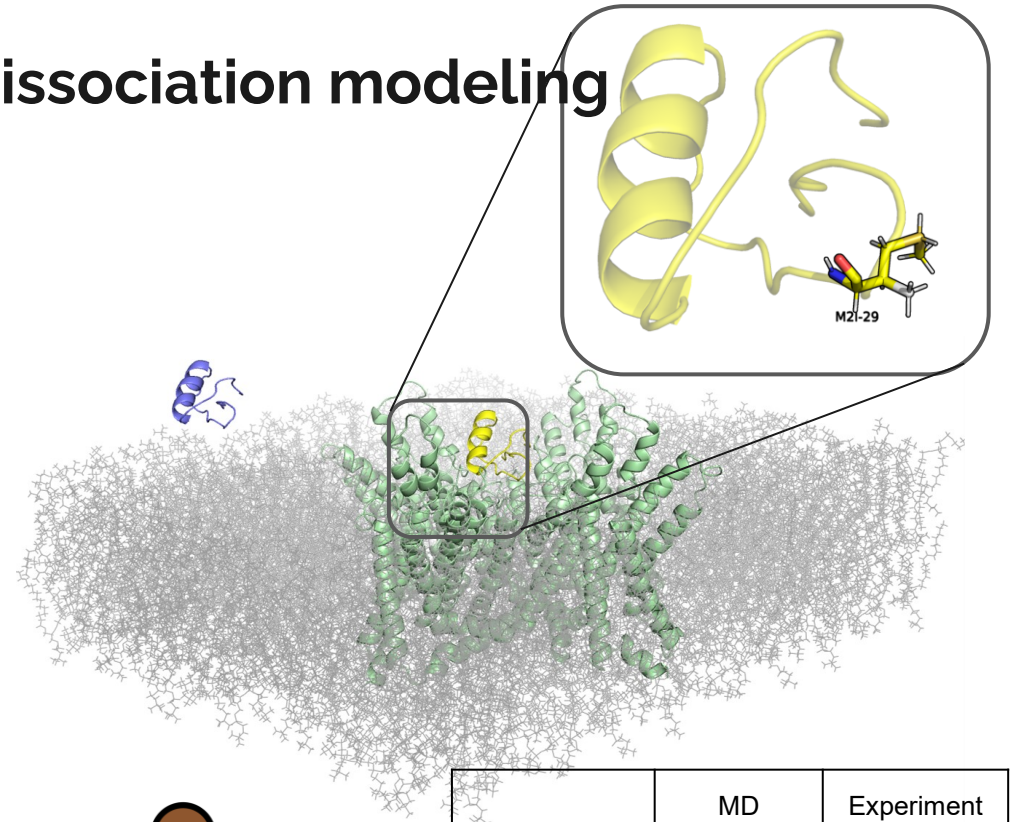




Alchemy: M→I without dissociation modeling



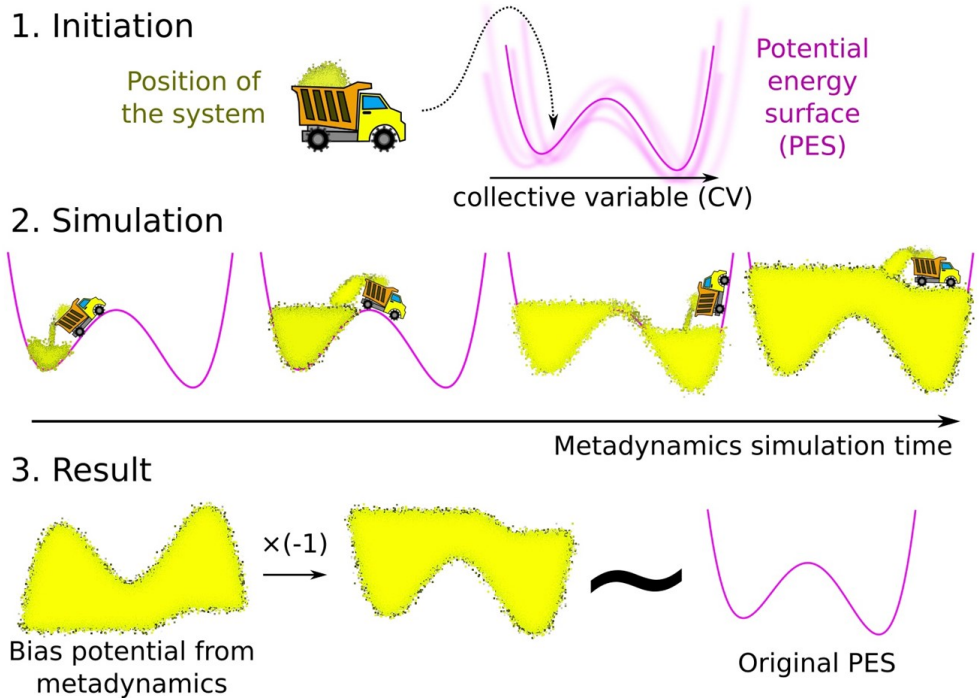
$$\Delta\Delta G^{Mutation}_{Binding} = \Delta G^{Mut}_{Binding} - \Delta G^{WT}_{Binding} = \Delta G^{Mutation}_{Unbound} - \Delta G^{Mutation}_{Bound}$$



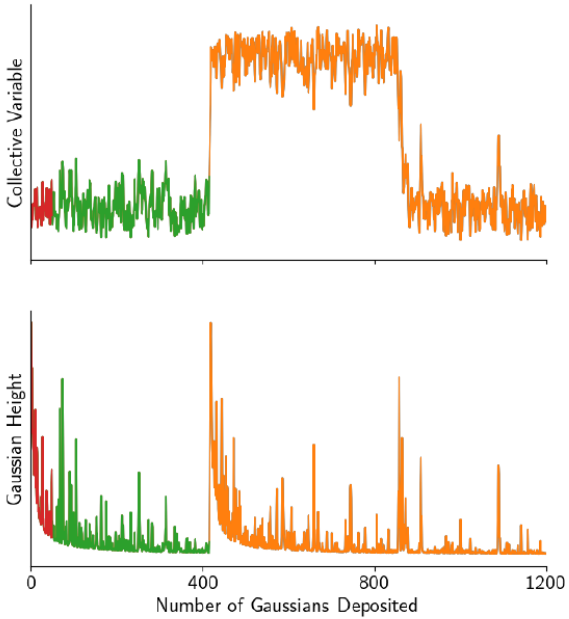
	MD (kcal/mol)	Experiment (kcal/mol)
MD 5 ns MD 7 ns	-0,04 -0,05	-4,33

Well-Tempered Metadynamics (WTMD)

"Metadynamics is a dynamics in the space of the CVs" [Parrinello 2006]



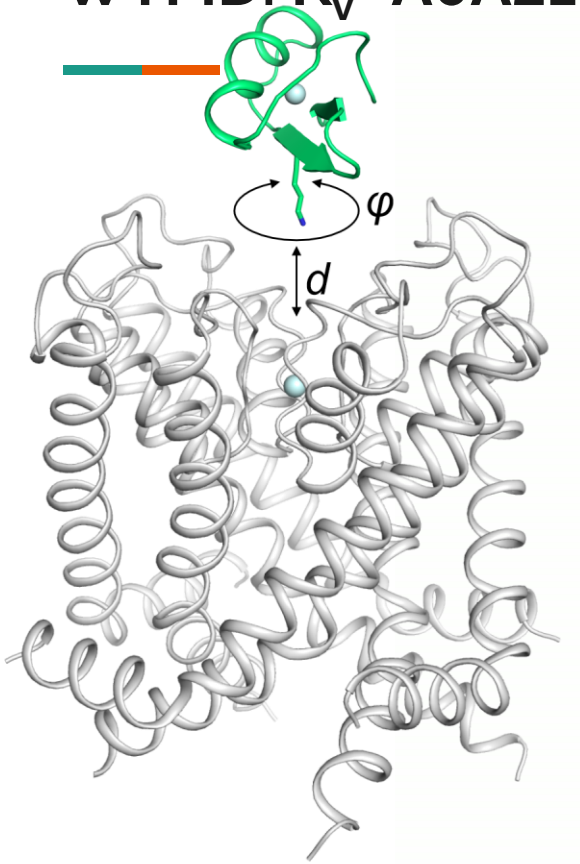
Metadynamics adds "hills" to the potential energy surface

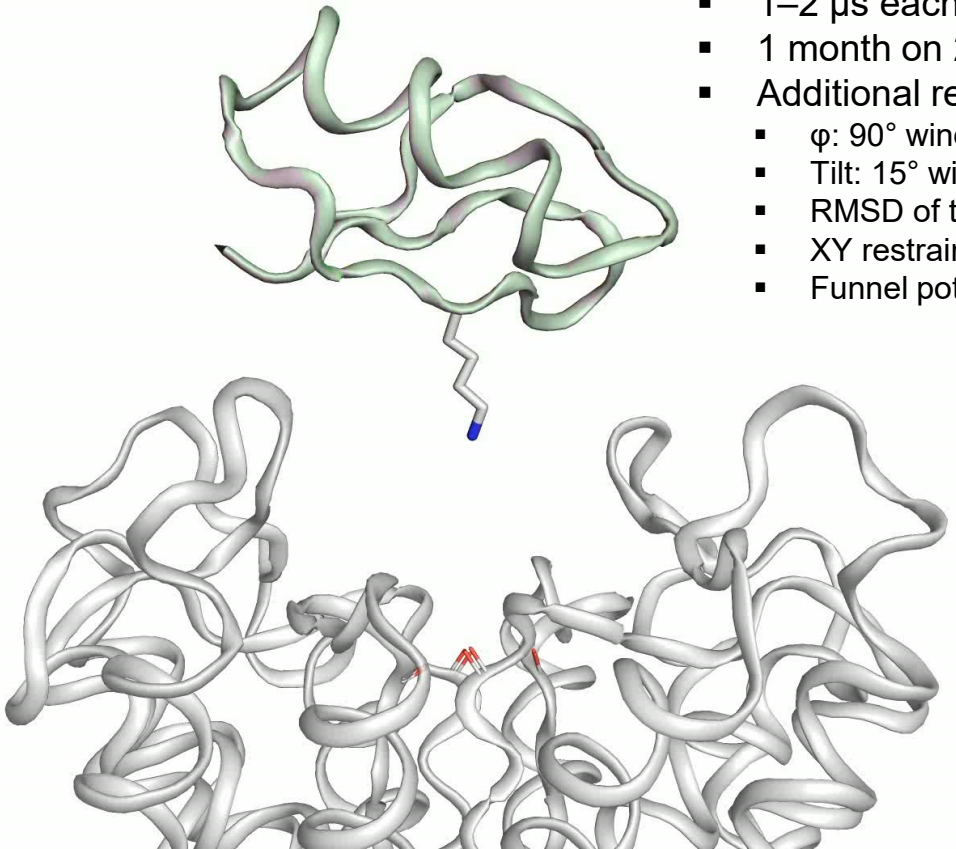


WTMD reduces hills height in a well-sampled regions

Time: 0 ns

WTMD: K_v–A0A218QXC2



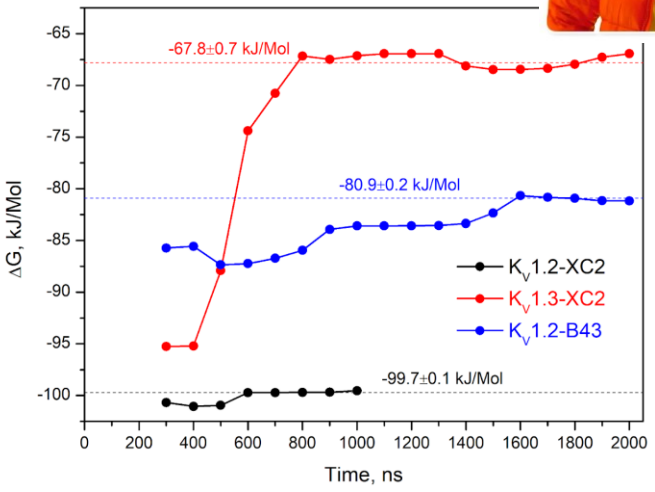


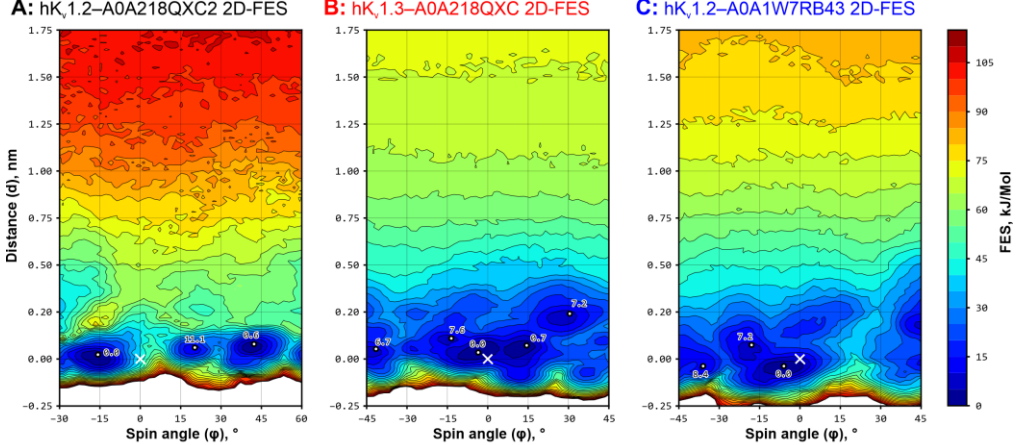
Calculation details:

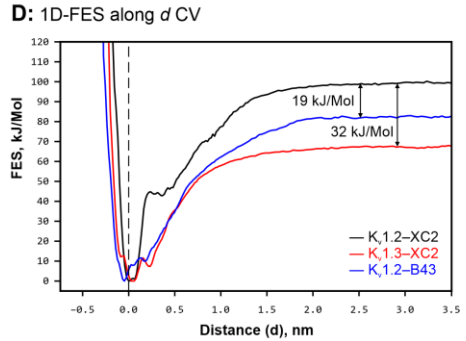
- 6-walker WTMD
- 1–2 μs each trajectory
- 1 month on 2×GPU node
- Additional restraints:
  - φ: 90° window
  - Tilt: 15° window
  - RMSD of toxin
  - XY restraint
  - Funnel potential

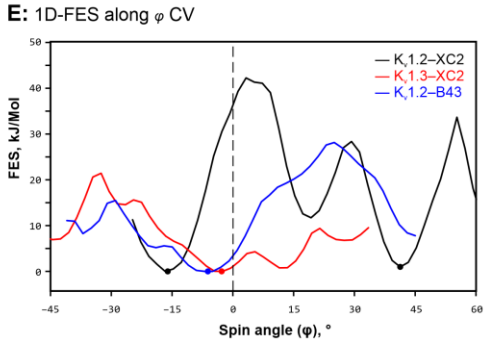
WTMD: realistic ΔΔG

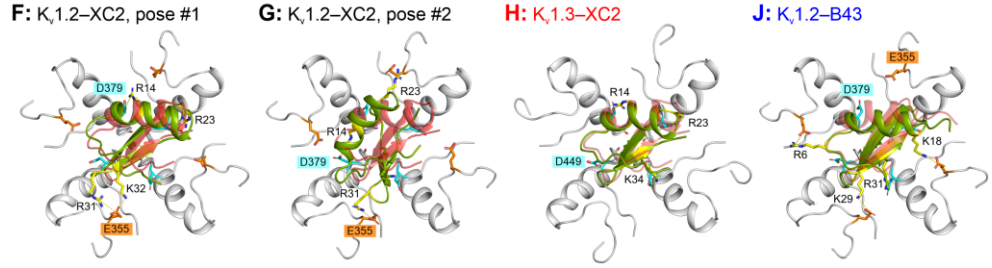
Convergence: 1+ μs needed











Conclusions

- Protein–protein interactions are hard to compute, but there are emerging physics-based methods
- Ensemble approach is critical for correct results
- Alchemical transformation: when complex *does not* change structure
- SOTA: well-tempered metadynamics
  - But: long, hard & expensive
  - Convergence is a key hurdle
- Emerging methods:
  - OneOpes (a Combined Enhanced Sampling Method to Rule Them All)
  - DeepTICA (AI-based method to search optimal CVs)