Efficient Binding Free Energy Estimation with Coarse-Grained Funnel Metadynamics

A. Grazzi[1], C.M. Brown[2], M. Sironi[1], S.J. Marrink[2], S. Pieraccini[1]

[1] Department of Chemistry, University of Milan, Italy

[2] Faculty of Science and Engineering, Rijksuniversiteit Groningen, The Netherlands

andrea.grazzi@unimi.it stefano.pieraccini@unimi.it

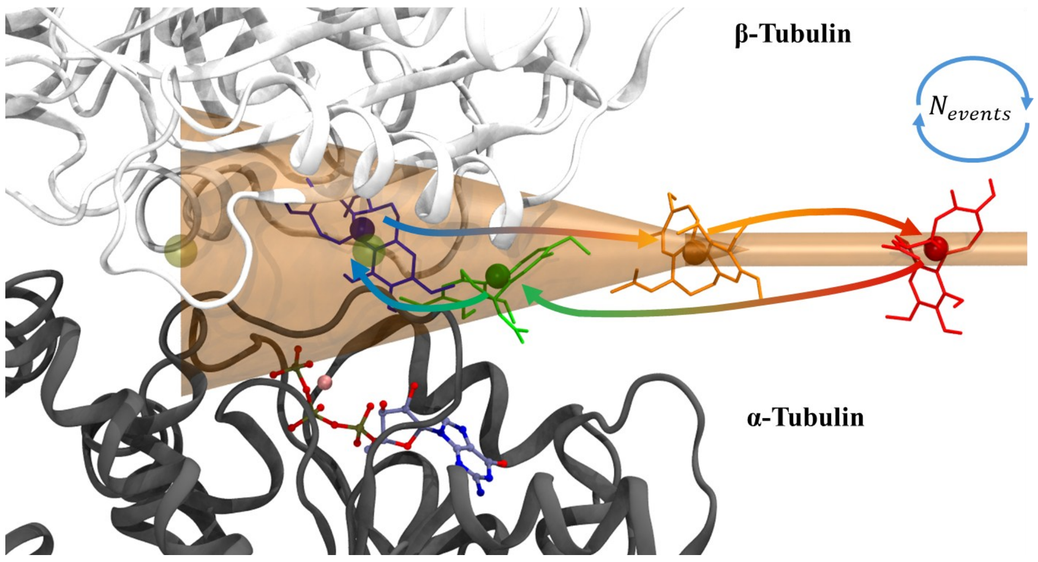

Figure 1. Restraining potential applied to the ligand's center of mass. The conical section of the funnel enables exploration of the colchicinoid binding site, while the cylindrical section prevents ligand dispersion into the solvent bulk. Coloured arrows indicate putative frames from a binding event.

Introduction

Accurate computation of protein-ligand binding affinities has long been an area of active interest in computational chemistry[1], as it can guide the drug design process. Classical high-throughput docking can screen many ligands cheaply, but its scoring functions are often oversimplified. In contrast, molecular dynamics (MD)–based free-energy methods explicitly include protein flexibility and solvent effects, potentially yielding more reliable affinities.

All-Atom Funnel Metadynamics (AA-FMD) has emerged as a powerful technique to characterize the binding process[2,3]. In particular, a funnel-shaped potential is applied to allow the ligand to explore orientations inside the binding pocket while restricting the roto-translation in the solvated state (**Fig.1**). Unfortunately, the high computational cost of atomistic simulations can hinder the scalability of this approach.

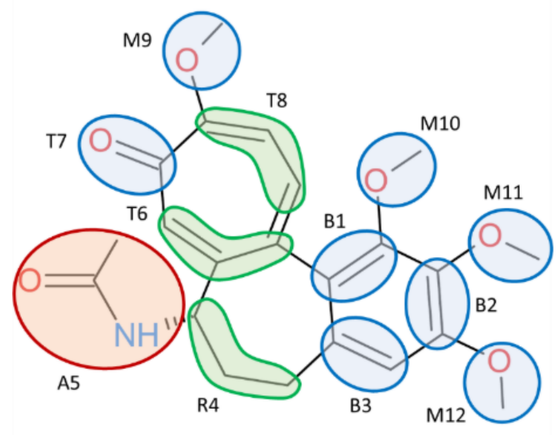

Figure 2. Mapping of colchicine to Martini3-resolution. Each bead *is characterized by a size and a chemical type, depending on the chemical fragment encompassed.*

Coarse-Grained Funnel Metadynamics

Coarse Grained Molecular Dynamics (CG-MD) allows to reduce the simulation cost by decreasing the number of particles simulated. The Martini3 FF is a well-established model that represents biomolecules and chemical moieties with CG-beads corresponding to chemical fragments (**Fig.2**). Protein-ligand interactions have been successfully investigated both in solution[4] and transmembrane environment[5].

In this work, we have assessed the feasibility of combining Funnel Metadynamics with the Martini3 FF to efficiently estimate binding free energies from CG-FMD simulations, while maintaining size-scalability.

Methodological validation

In order to assess the validity of the **methodology**, we simulated the binding process of the compound colchicine to two model proteins (**Colchicalin**,**BRD4**)(**Fig.3**). We have assessed the **robustness** of the ΔG_{bind}^{CG-FMD} prediction with respect to various aspects of the simulation setup. For instance, the role of the CG-backbone model (i.e. EN, OLIVES, goMartini) has been explored, highlighting the crucial impact of **protein flexibility** (**Fig.4**). Furthermore, we observed that the **funnel size doesn't affect** ΔG_{bind}^{CG-FMD}.

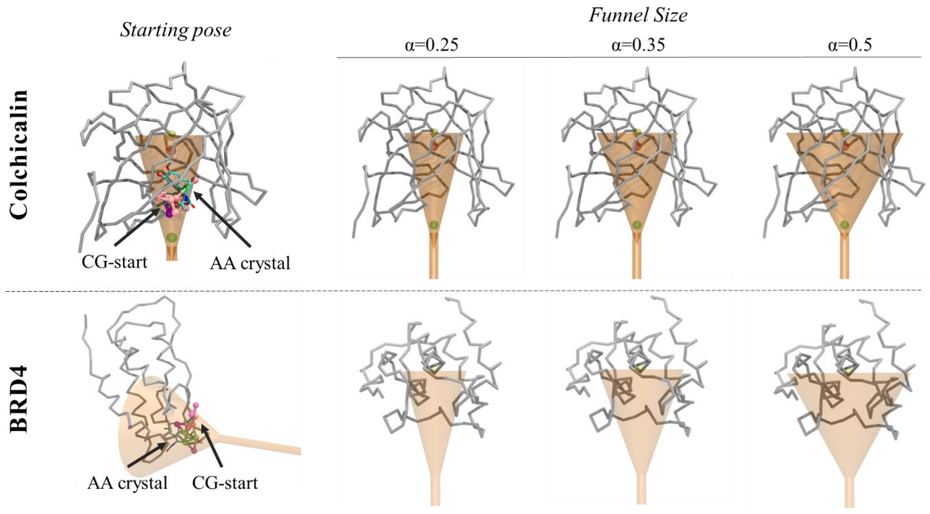

Figure 3. Model proteins investigated with FMD; Top row: Colchicalin (pdb 5NKN). Bottom row: BRD4 (pdb 4LZR).

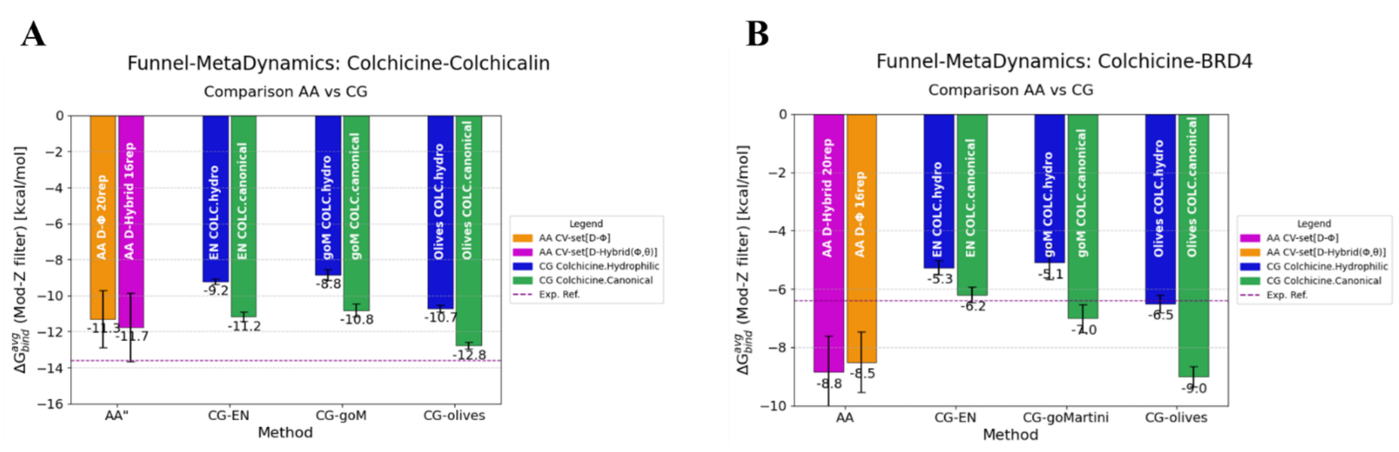

Figure 4. Comparison of computational ΔG_{bind} with experimental reference (dashed line) for the binding of colchicine with A) Colchicalin and B) BRD4. On the left-side of each plot, two ΔG_{bind}^{AA-FMD} estimates are reported. On the right side, three sets of ΔG_{bind}^{CG-FMD} predictions are reported.

Simulation Efficiency

The improved efficiency of CG-FMD compared to AA-FMD has been determined by computing the **average** number of **binding events** observed in **1μs** at both resolutions. Interestingly, CG-FMD can capture **50%** more binding events (**Fig.6**). Furthermore, if we compare the rate at which new binding events are observed (as a function of the simulation wall-time), CG-FMD can offer an improvement of **25 times** compared to AA-FMD, with the additional advantage of requiring less hardware.

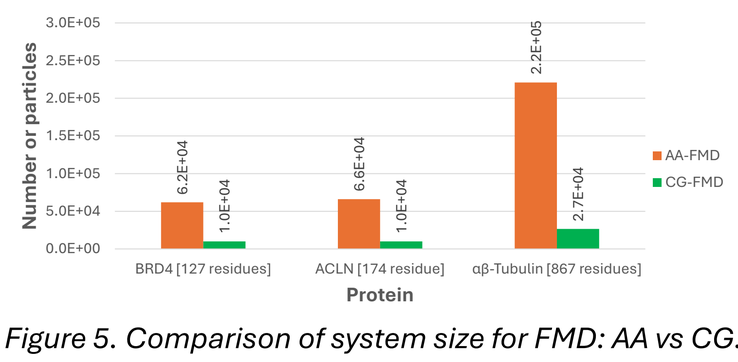

Figure 5. Comparison of system size for FMD: AA vs CG.

Simulation type	CPU-allocation	GPU-type	Performance	#Binding Events/ 1μs-simulation	Walltime 1μs-simulation	Rate of acquisition [Events/h]
AA-FMD	8cores-Intel Xeon Platinum 8358@2.60GHz	NVIDIA Ampere A100 64GB	190ns/day	15.0	**126.3 h**	0.1
CG-FMD	8cores-AMD EPYC 7763@2.45 GHz	***none***	3600ns/day	22.8	**6.7 h**	3.4

Figure 6. Hardware requirements and rate of binding event collection: AA-FMD vs CG-FMD; Data from the Colchicine-Colchicalin system.

KEY-takeaways

- CG-FMD **scales well** with system's size (**Fig.5**).
- CG-FMD is **25x more efficient** than AA-FMD.

Towards microtubule pharmacology

Extension of this simulation approach to a strategic pharmacological target, i.e. the **tubulin αβ-heterodimer**, is ongoing. The crystallographic binding site of colchicine is quite buried (**Fig.1**), therefore FMD is required to model the entire binding process. Interestingly, CG-FMD can estimate a ΔG_{bind}^{CG-FMD} that is comparable to experimental references (**Fig.7**).

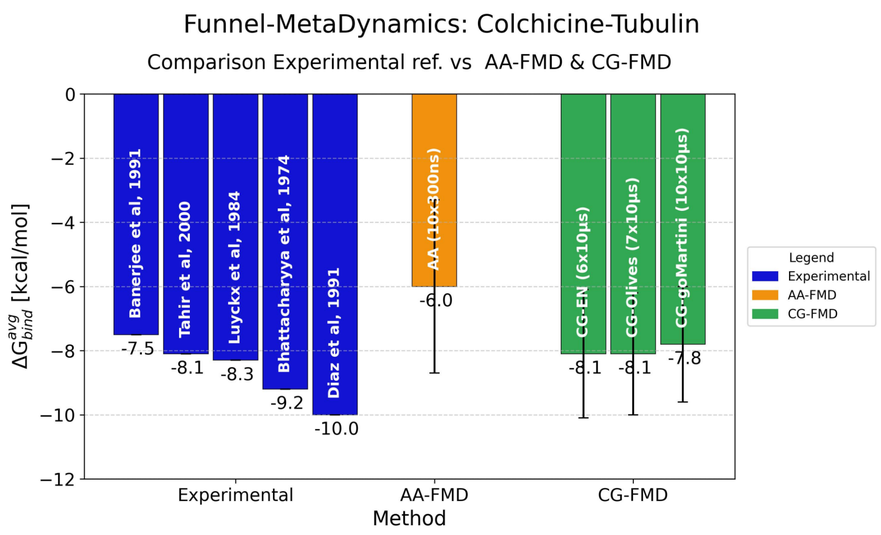

Figure 7. Colchicine-Tubulin ΔG_{bind} estimates: on the left, experimental references. Middle bar: AA-FMD estimate. On the right: CG-FMD predictions

References

1. Limongelli, V., *Wiley Interdisciplinary Reviews Computational Molecular Science*, 2020, **10**
2. Limongelli, V. B., *PNAS*, 2013, **110**, 6358-6363.
3. Raniolo, S., & Limongelli, V., *Nature Protocols*, 2020,**15**, 2837-2866.
4. Souza, P. C. T., ... , Raniolo, S., Limongelli, V., & Marrink, S. J. , *Nature Communications*, 2020, **11**,3714
5. Bartocci, A., Grazzi, A., Awad, N., Corringer, P., Souza, P. C. T., & Cecchini, M., *Nature Communications*, 2024, **15**, 9040.