Development of a Coarse-Grained Molecular Dynamics (CG-MD) approach to explore tubulin-ligand binding

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Introduction

Microtubules (MTs) regulate several cellular functions including intracellular cargo trafficking and cell proliferation. They are highly dynamic polymers composed of linear assemblies of the tubulin αβ heterodimer. MTs play a significant role during cell mitosis and pharmaceutical research has developed several compounds acting as Microtubule Targeting Agents (MTAs) to modulate their behaviour. Indeed, several tubulin-ligands are known, and eight distinct binding sites have been experimentally identified[1].

Current computational investigations of tubulin-ligand interaction with All-Atom Molecular Dynamics (AA-MD) are limited by the high computational cost. In recent years, Coarse-Grained Molecular Dynamics has emerged as a possible alternative which benefits from a speed-up of three orders of magnitude[2]. In particular, the Martini3 model has been shown to accurately reproduce binding pose and affinity of small molecules for proteins for a limited set of test systems[3].

Aim

This project aims to assess whether the Martini3 model is suitable for investigating tubulin-ligand binding by performing unbiased CG-MD simulations, where the ligand is placed in the solvent bulk and is left free to approach the protein.

To this end, a collection of known binders of pharmaceutical interest will be parameterized to the CG resolution of the Martini3 model.

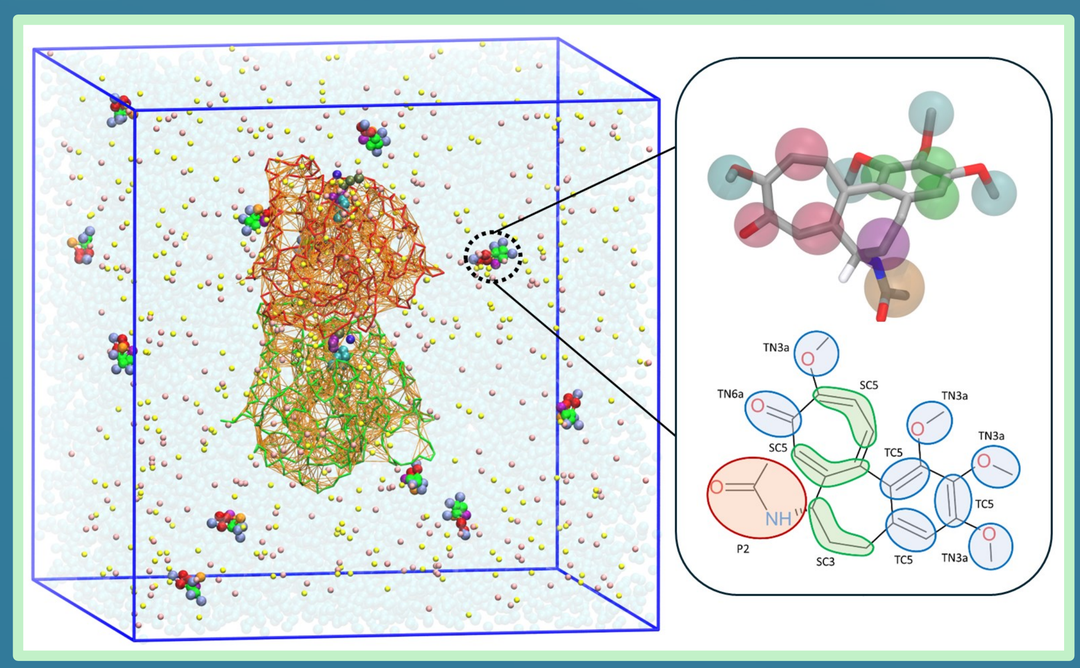
The analysis of the simulation will locate the binding pocket and characterize the most favourable poses within the framework of this computational approach.

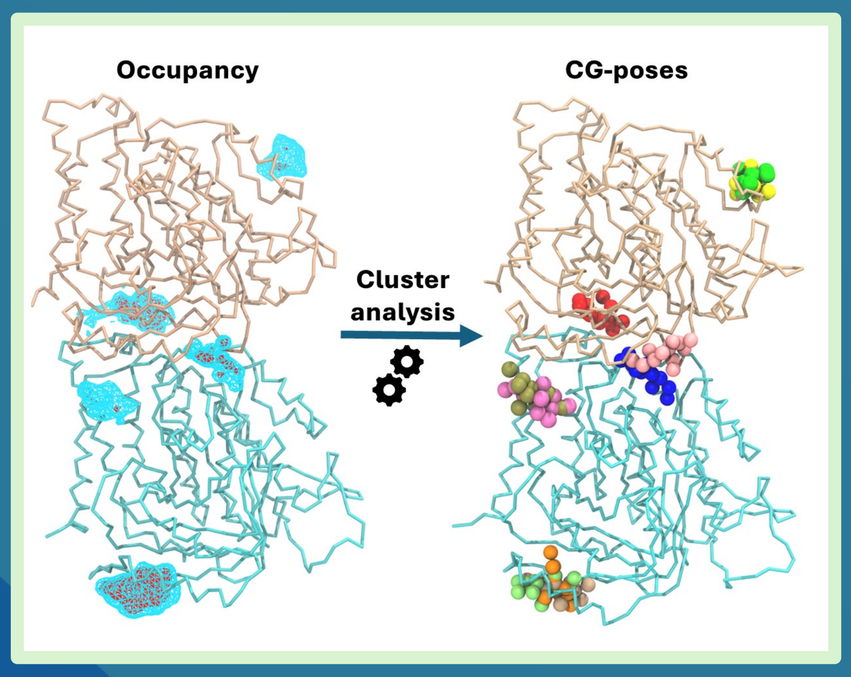
A comparison with the available crystal structures will be used to assess whether the CG-predictions are correct or if further refinements with more accurate methods should be introduced in the computational protocol to reproduce the experimental findings.

Coarse-Grained Molecular Dynamics

The first step of the investigation has been the parameterization of colchicine, a well-known tubulin ligand, to the Martini3 model. Several CG-MD simulations of the tubulin αβ-heterodimer in explicit solvent have been performed. An Elastic Network (EN) has been imposed on the tubulin dimer to maintain the structure. A snapshot of the simulation box is reported in Figure 1. Thanks to the reduced computational cost, 300 μs of simulation have been collected.

Figure 1. On the left, simulated biosystem at the CG resolution. Tubulin dimer is represented with licorice). Colchicine molecules are depicted with beads. On the right: top, overlay of atomistic structure of colchicine with CG beads; bottom, mapping of the 2D structure and bead type assignment.





Occupancy & Cluster analysis

The starting point for the analysis of the simulations has been the computation of occupancy isocurves for colchicine on the proteins surface. The simulated timescales allow for numerous ligands' binding/unbinding events. Therefore, several possible pockets are identified.

By performing a cluster analysis on long-lived binding events (lasting at least 100ns), the ten most probable CG bound poses have been selected. Interestingly, five of them are located at the interface between the two subunits, approaching the experimentally observed binding site.

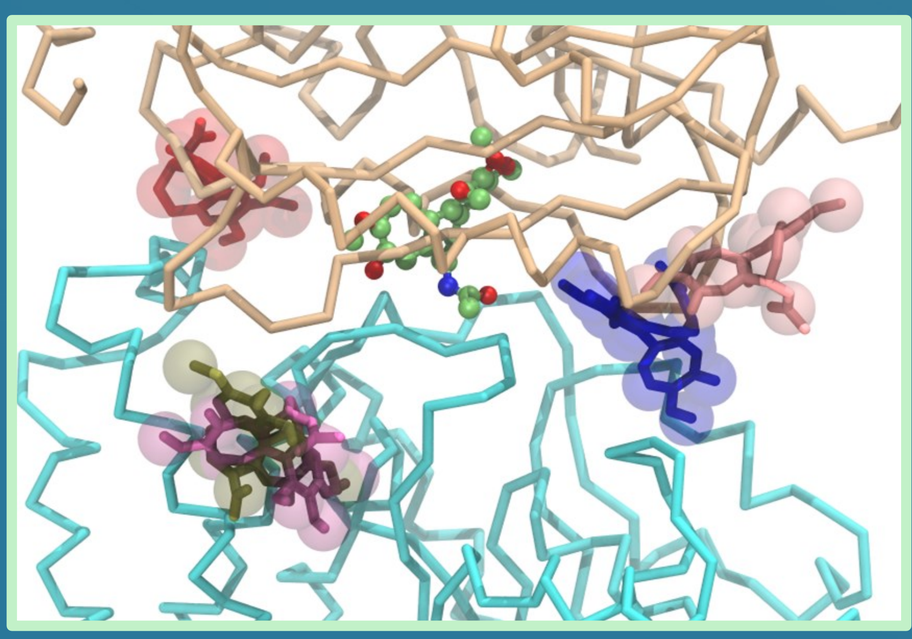
Figure 2. Analysis of CG-MD simulation. On the left, occupancy isocurves have been computed with VMD. On the right, the ten most relevant CG-poses have been identified with a cluster analysis.

Conversion to All-Atom resolution

Thanks to a procedure known as backmapping, it is possible to recover the fully atomistic description of the system starting from a CG configuration with the CG2AT tool[4]. This backmapped AA structures can be used for direct comparison with the experimental reference and as initial configuration for in-silico refinement with more accurate methods, such as AA-MD. In this way, the most relevant CG-poses efficiently obtained without a priori information can be used as a reasonable starting point for enhanced sampling methods.

Figure 3 represents the five most relevant CG poses closest to the experimentally observed binding site of colchicine converted to AA resolution.

Figure 3. Backmapping of the most relevant CG-poses to All-Atom resolution. CG poses are represented as transparent beads, AA poses are depicted with licorice. Experimental pose of colchicine is represented as green ball & stick.



Future objectives

Starting from these preliminary results, the next step will be to apply enhanced sampling approaches from the relevant poses identified, trying to elucidate the complete binding pathway of colchicine to its experimental binding pocket. In addition, parameterization of additional ligands of pharmacological interest, targeting more solvent-exposes pockets, is underway.

References

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