Integrative modelling for the characterisation of the structure and dynamics of biomolecules

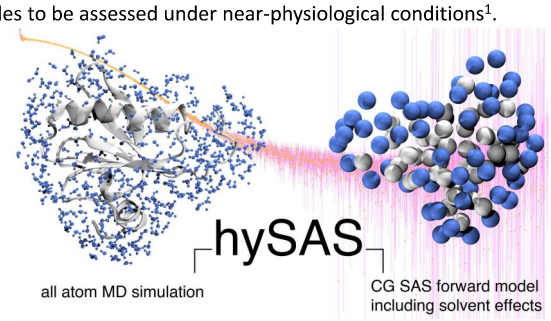
DIPARTIMENTO DI BIOSCIENZE
UNIVERSITÀ DEGLI STUDI DI MILANO

Federico Ballabio @CamilloniLab | federico.ballabio@unimi.it

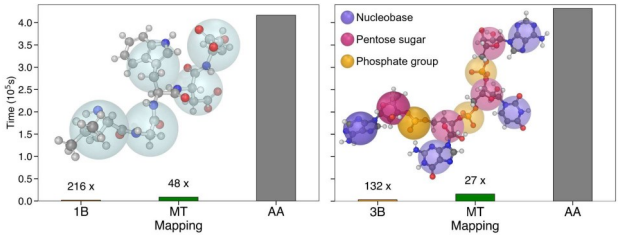
Combining experimental data with computational methods is an established and successful strategy for characterising the structure and dynamics of biomolecules. This integrative approach improves both the predictive capabilities and the accuracy in the description of molecular mechanisms to the atomistic scale. In this work, we present three projects that highlight the potential of this strategy across diverse disciplines.

1. An accurate and efficient SAXS/SANS implementation including solvation layer effects suitable for molecular simulation.

Small Angle Scattering is a low-resolution technique based on X-rays (SAXS) or neutrons (SANS) that allows the size, shape, stoichiometry, and dynamics of biomolecules to be assessed under near-physiological conditions[1].

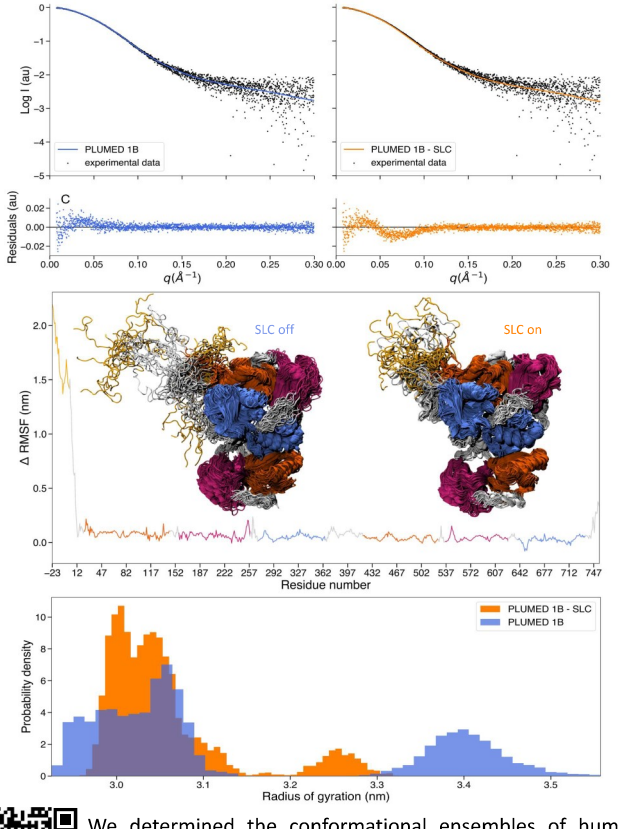


Our approach consists in restraining all-atom molecular dynamics (MD) simulations with SAS data using a coarse-grained (CG) forward model that reproduces an amino acid with a single bead (1B) and a nucleotide with three beads (3B). The beads exposed to the solvent are corrected on-the-fly to include solute-solvent scattering effects (SLC) at no additional computational cost.



The forward model performance is evaluated at different resolutions: all-atom (AA), Martini (MT), 1B per amino acid / 3B per nucleotide.

- 6,500 frames of gelsolin: 11,558 atoms (AA), 1,627 MT beads, 775 1B beads.
- 500 frames of ribosome RNA: 38,287 atoms, 7,796 MT beads, 3,560 3B beads.

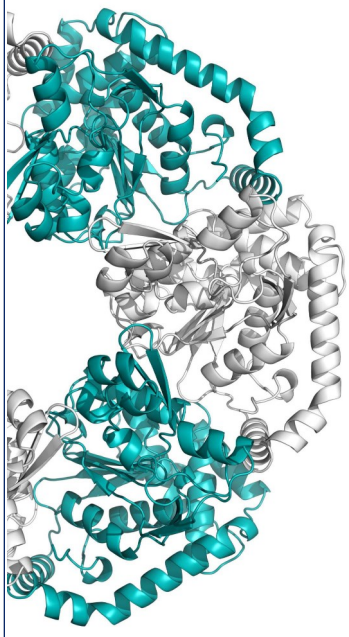


We determined the conformational ensembles of human full length gelsolin with and without the solute-solvent interactions. Including the SLC leads to more compact and stable models.

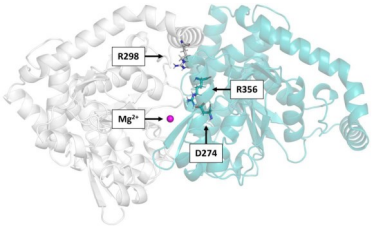
- SLC on: average RG of 3.05 nm, RMSF (residues) of 0.26 nm
- SLC off: average RG of 3.14 nm, RMSF (residues) of 0.38 nm

2. Structural characterisation of the barley pale green mutant *TM2490*.

The mutation responsible for the *TM2490* phenotype has been identified in the *ChlI* ATPase subunit of magnesium chelatase, an enzyme that catalyses the insertion of magnesium into protoporphyrin IX and leads to chlorophyll synthesis[2].

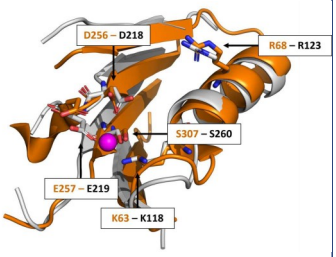


- The ATPase belongs to AAA+ superfamily.
- No solved structures are available to date.
- The mutation consists in R298K.

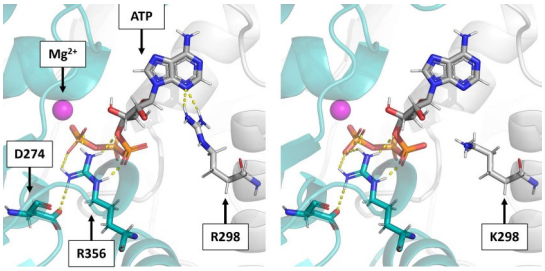


We generated the model using Alphafold2 Multimer and Schrödinger suite:

- R298 is located in the ATP-binding cleft, at the interface of two monomers. R298K does not lead to the loss or formation of intra- or inter-monomer contacts.
- D274 (*Chlorina 125*) and R356 (*Chlorina 157*) establish intra-monomer h-bond network which includes R393.



The homology between the ATP-binding domain of Heat Shock Locus U (HSLU - PDB 1DO0) and the ChlI barley model allowed us to infer the distances between the Mg^{2+} ion and the conserved residues in both structures. The ATP was docked into the binding cleft of the model using the Mg^{2+} ion as a restraint.

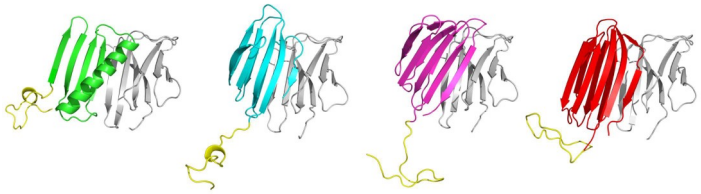


R298 could establish hydrogen bond interactions with ATP (left panel). The same interaction could be affected or reduced by the mutation, due to the shorter side chain and the presence of only one amino group in lysine (right panel). This hypothesis supports the experimental results, which show that the mutation does not reduce the assembly and accumulation of ChlI in leaves, but its activity.

Acknowledgements: group of Prof. P. Pesaresi.

3. *De novo* binders design for beta-2 microglobulin.

Beta-2 microglobulin (B2M), a component of MHC-I complex, is involved in the progression of multiple myeloma[3]: B2M molecules aggregate in fibrils into macrophages lysosomes and the damage leads to the secretion of pro-inflammatory cytokines. Trying to rationalizing possible binding mechanisms to B2M, we generate models of B2M binders with RoseTTAFold Diffusion[4]. The models were modified to include His-tag and the stability was assessed with MD.



Acknowledgements: group of Prof. S. Ricagno.

REFS: 1.Tuukkanen et al. 2017, *IUCrJ* | 2.Gao et al. 2020, *Protein Science* | 3.Hofbauer et al. 2021, *Immunity* | 4.Watson et al. 2023, *Nature*