Mcl-1 conformational landscape exploration

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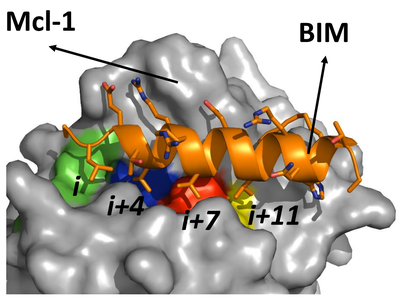
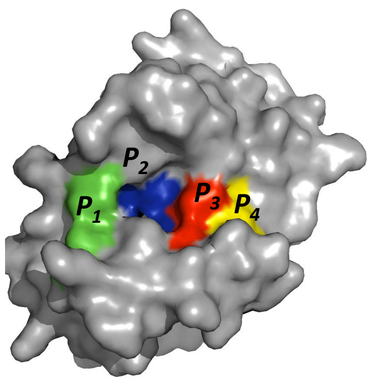
Abstract: In this study, we explored the structural dynamics of Mcl-1: an anti-apoptotic protein.

Mcl-1 an anti-apoptotic protein

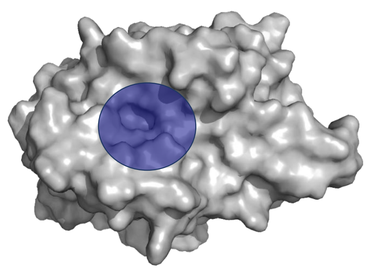
Myeloid cell leukemia sequence 1 (MCL-1) is an anti-apoptotic member of the BCL-2 family. Apoptosis is regulated by complex interactions between pro- and anti-apoptotic proteins in the BCL-2 family. MCL-1 inhibits apoptosis by directly binding the pro-apoptotic effector proteins BAK and BAX, and pro-apoptotic BH3-only proteins, such as BIM.

In the case of peptide BH3-partenrs partners, four conserved hydrophobic residues at positions i, i+4, i+7 and i+11 on the contact face of the α helical peptide are projected into the hydrophobic cavities of Mcl-1 binding groove. The receiving cavities of these four hydrophobic residues are usually named P1(i), P2(i+4), P3(i+7) and P4(i+11) respectively.

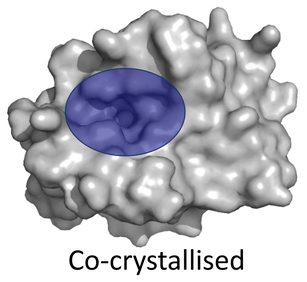




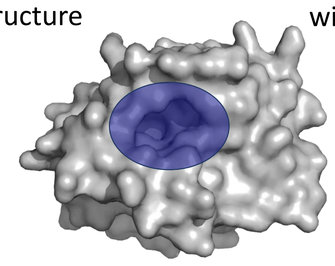
The solved X-ray structures have allowed to distinguish three different conformational states of Mcl-1



APO-structure



Co-crystallised with peptide



Co-crystallised with synthetic ligand

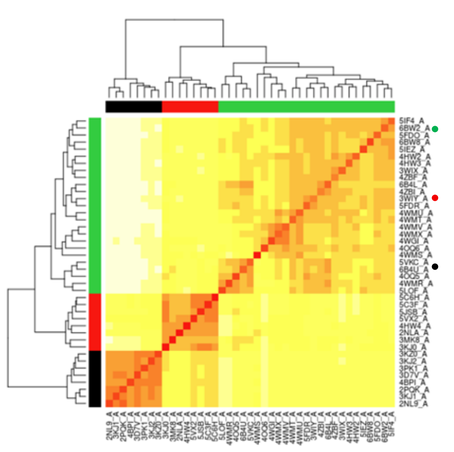
The synthetic ligands bind in the same binding groove as BH3-only partners. Nevertheless, we should highlight the high plasticity of the binding pockets, especially the P2 and P3 pockets which are the main pockets targeted by the synthetic ligands.

The aim of this work was three-fold:

1. Propose a better understanding of the key structural elements leading Mcl-1 to adapt to its different binding partners using ensemble structures and essential dynamics;
2. Quantify the breathing motion at the binding interface of Mcl-1 and provide a free energy surface that better describes the likelihood of the conformational states explored by Mcl-1 in solution;
3. Provide a detailed understanding of how Mcl-1 allosteric inhibition works, by exposing the conformational population shift and highlighting the existence of an allosteric communication network through pocket crosstalk analysis.

The hierarchical clustering

Mcl-1 conformational space derived from the X-ray structures dataset (comprising 41 Mcl-1 X-ray structures) reveals three main conformational ensembles from the hierarchical clustering (hclust) analysis:



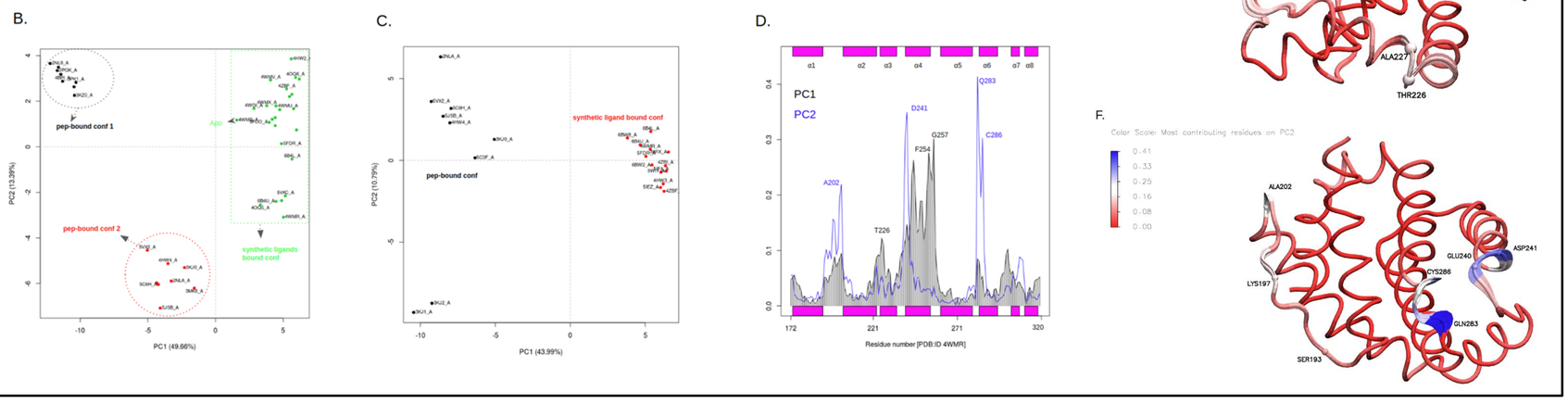
- APO and synthetic-ligand
- 1st cluster of peptide-bound
- 2nd cluster of peptide-bound

Principal component analysis

In order to highlight the structural differences between the conformers from an ensemble point of view, we performed a PCA:

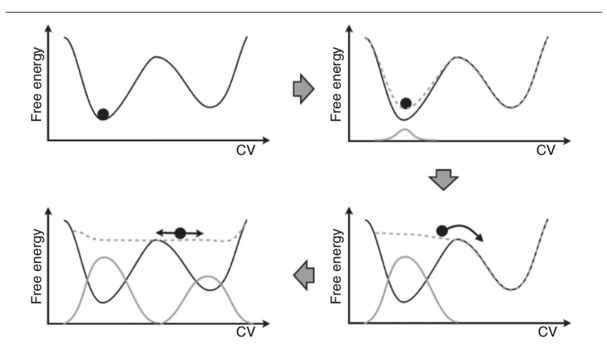
the full dataset (41 Mcl-1 X-ray structures)

the reduced dataset (comprising only structures with no missing residues)

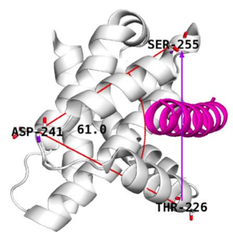


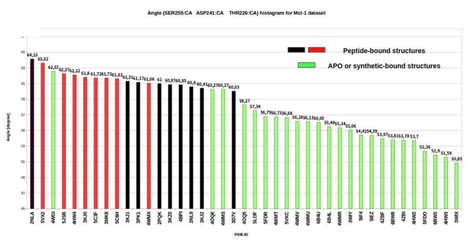
Metadynamics

Metadynamics is a powerful technique for enhancing sampling in molecular dynamics simulations and reconstructing the free-energy surface as a function of few selected degrees of freedom, often referred to as collective variables (CVs).

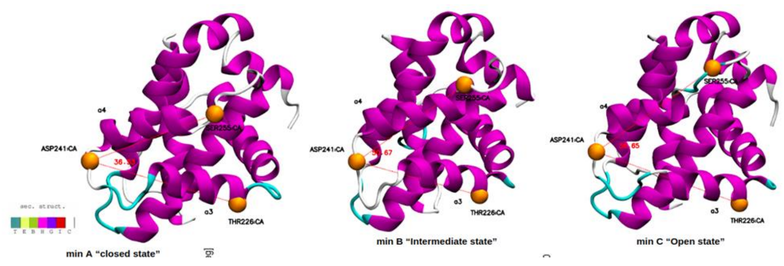


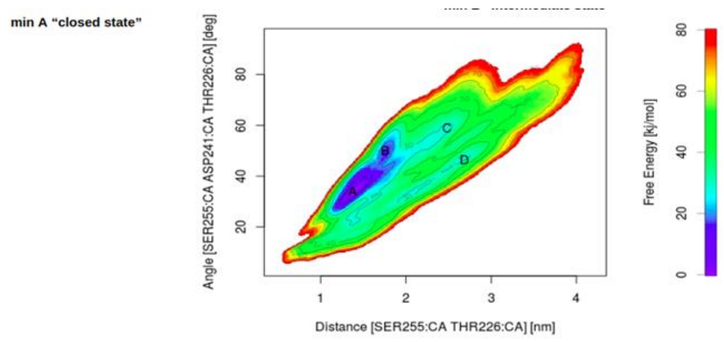
Collective variables selected for the Metadynamics





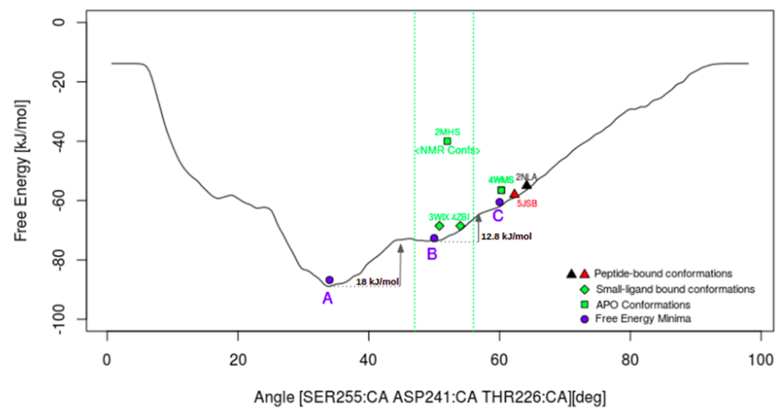
The free-energy surface of Mcl-1's breathing motion and most populated free-energy wells. Representative structures for the three main states (A, B, C) are shown on top; colors represent the secondary structures using the STRIDE color scheme. Cα-atoms forming the angle [Ser255-Asp241-Thr226] are represented in VDW spheres and colored in orange.





To assess the reliability of our metadynamics simulation the free-energy wells should correspond to energetically favored conformations.

Free-energy profile as a function of the CV angle [Ser255-Asp241-Thr226]. Free-energy wells are represented in blue circles, APO models in green squares, and peptide-bound structure in black and red triangles. The angle domain [min, max]=[47°,56°], spanned by the Mcl-1 APO NMR models [PDB ID: 2MHS] is represented in green dashed lines.



Minimum A (centered around 34°) - corresponds to the closest conformation and it is not yet covered by the experimentally available data

Minimum B - corresponds to an intermediate state 'ready to bind' with an angle varying from 47° to 56°. It spans the domain covered by the only NMR APO ensemble currently available for hMcl-1 (PDB ID: 2MHS) and covers as well the synthetic-ligands conformations.

Minimum C - corresponds to a more open conformation, very similar to what the peptide-bound conformations adopt, we consider it as a transition state towards a more deep free-energy well, that might be induced and stabilized by the binding with a peptide.

References:

M.Benabderrahmane et al. J Chem Inf Model. 2020 Jun 22;60(6):3172-3187. doi: 10.1021/acs.jcim.0c00315. Epub 2020 May 28.