Molecular Dynamics of Bcl-2 with Ligands

June 3, 2022

1 Data

- 2XA0: Crystal structure of BCL-2 in complex with a BAX BH3 peptide

2 Bcl-2 gene [Gomez2019]

- The Bcl-2 gene can potentially encode two proteins: Bcl-2α and Bcl-2β (239 and 205 amino-acid residues in length, respectively) [13–15].
- Bcl-2α contains a transmembrane domain, which is absent in Bcl-2β.
- The NMR structure of Bcl-2α (PDB 1G5M) consists of eight α-helices. Two of these (α5 and α6) form the hydrophobic core of the protein surrounded by four amphipathic helices (α1, α2, α3, and α4).
- Commonly, Bcl-2 (similarly to the rest of the Bcl-2 family members) is described by its BH domains. Bcl-2 contains four BH domains; BH1, BH2, BH3, and BH4 (residues 139–157, 190–206, 100–109, and 14–29, respectively). BH1, BH2, and BH3 form a hydrophobic pocket, which binds BH3 domains of other family members, thereby establishing interactions. BH4 does not participate in protein dimerization, but it may be necessary for anti-apoptotic activity.
- Bcl-2 also contains an intrinsically disordered region (IDR) located between α1 and α2 and which is composed of 58 amino-acid residues (Gly33-Ala91), which we denote here as a flexible loop domain (FLD).
- Traditionally, IDRs were considered to be passive segments in protein sequences that "linked" structured domains, but now, we know that these regions are highly flexible to facilitate conformational adaptability, and in consequence, to bind different types of ligands.
- The FLD structure has not been resolved. Nevertheless, due to its importance in the function of Bcl-2, it has been analyzed by molecular dynamics simulations (MDS) in order to describe its flexibility and stability [18].
- Additionally, the ninth α-helix at the C-terminus (Leu217-Lys239) of Bcl-2α constitutes the transmembrane domain (TMD).
- It has been established that the Bcl-2 proteins require the TMD to retain their full anti-apoptotic activity. As a consequence, Bcl-2β proteins lacking the TMD reside in the cytoplasm [13]. There is insufficient information regarding the contributions of the TMD region to the overall conformational dynamics and membrane targeting ability of Bcl-2, as most in silico and in vitro studies have been performed with constructs lacking the TMD [19,20].

3 Structure of full-length BCL-2 [Lan2020]

- BCL-2 is a tail-anchored protein. The structure of full-length BCL-2 (Fig. 1) contains both a C-terminal hydrophobic helix (α8) that functions as a transmembrane (TM) domain and a 65-residue-long highly flexible loop domain (FLD) located on the opposite side of its ligand-binding groove.

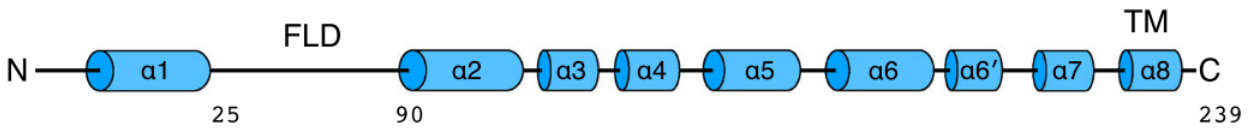

Figure 1: Cartoon representation (helices shown as cylinders) of full-length BCL-2.

- The structure of full-length BCL-2 protein has not been determined, but there have been reports of various truncated BCL-2 structures (e.g., PDB codes: 2XA0, 1GJH, 4MAN), all of which lack the FLD and TM (Fig. 2).

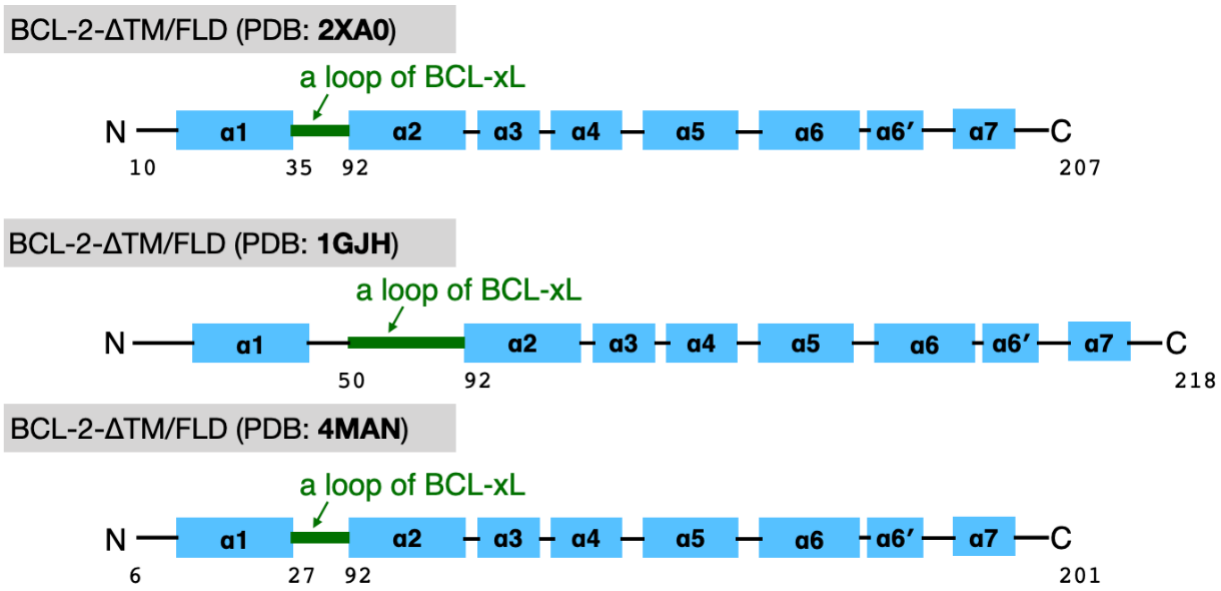

Figure 2: Various truncated BCL-2 structures (e.g., PDB codes: 2XA0, 1GJH, 4MAN), all of which lack the FLD and TM.

4 Membrane-integrated conformations

"Two different membrane-integrated Bcl-xL conformations have been identified: membrane-inserted and membrane-anchored Bcl-xL. The former is characterized by extensive refolding [15,16], while the latter retains the fold of the soluble state (aside from the released α8 anchor helix) [12–14]." [Tyagi et al., 2021, Tyagi2021].

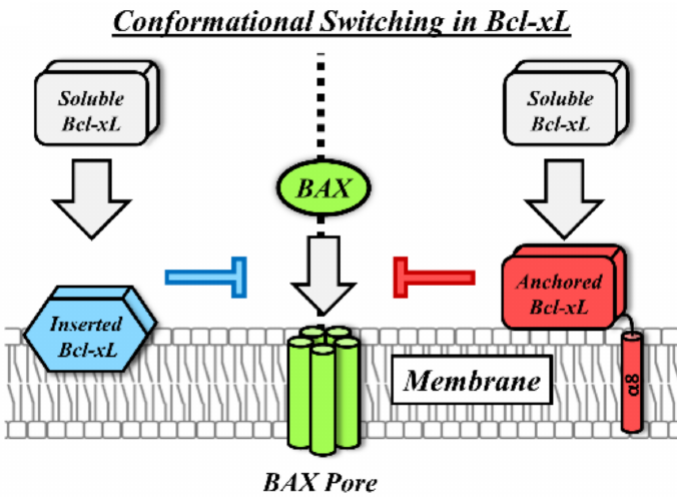

Figure 3:

4.1 Anchored Bcl-xL

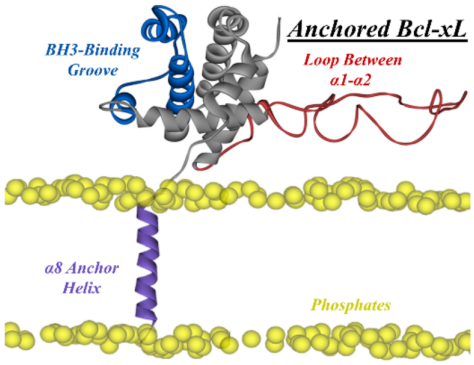

Figure 4: [Tyagi et al., 2021, Tyagy2021]

4.2 Membrane-embedded

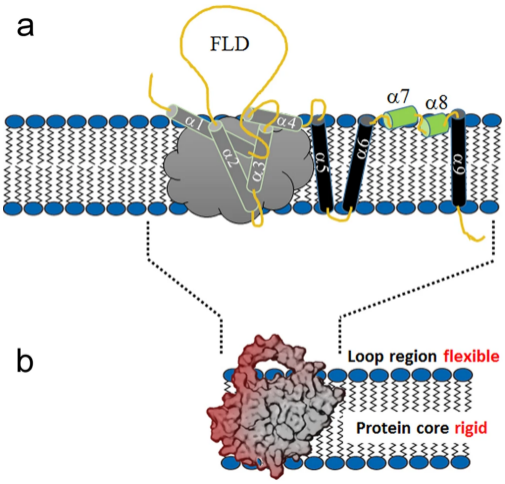

Figure 5: [Mushtaq et al., 2021, Mushtaq2021]

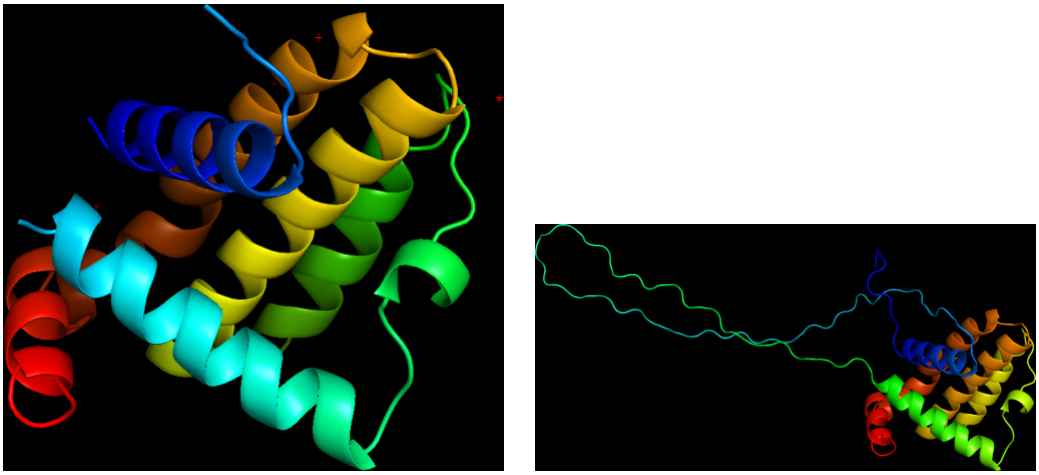

Figure 6: Flexible loop domain (FLD) reconstruction using Modeller.

5 Bcl-2 (PDB 2xa0)

6 Bcl-2 and Bcl-Xl

“Since the amino acid sequences of these proteins are very similar, the three-dimensional structures of both proteins are also similar, implying that inhibitors that can bind to Bcl-2 may also bind to Bcl-XL”. [Wakui et al., 2018, Wakui2018]

References

[Mushtaq et al., 2021] Mushtaq, A. U., Åden, J., Clifton, L. A., Wacklin-Knecht, H., Campana, M., Dingeldein, A. P. G., Persson, C., Sparrman, T., and Gröbner, G. (2021). Neutron reflectometry and NMR spectroscopy of full-length Bcl-2 protein reveal its membrane localization and conformation. *Communications Biology*, 4(1):507.

[Tyagi et al., 2021] Tyagi, V., Vasquez-Montes, V., Freites, J. A., Kyrychenko, A., Tobias, D. J., and Ladokhin, A. S. (2021). Effects of Cardiolipin on the Conformational Dynamics of Membrane-Anchored Bcl-xL.

[Wakui et al., 2018] Wakui, N., Yoshino, R., Yasuo, N., Ohue, M., and Sekijima, M. (2018). Exploring the selectivity of inhibitor complexes with Bcl-2 and Bcl-XL: A molecular dynamics simulation approach. *Journal of Molecular Graphics and Modelling*, 79:166–174.

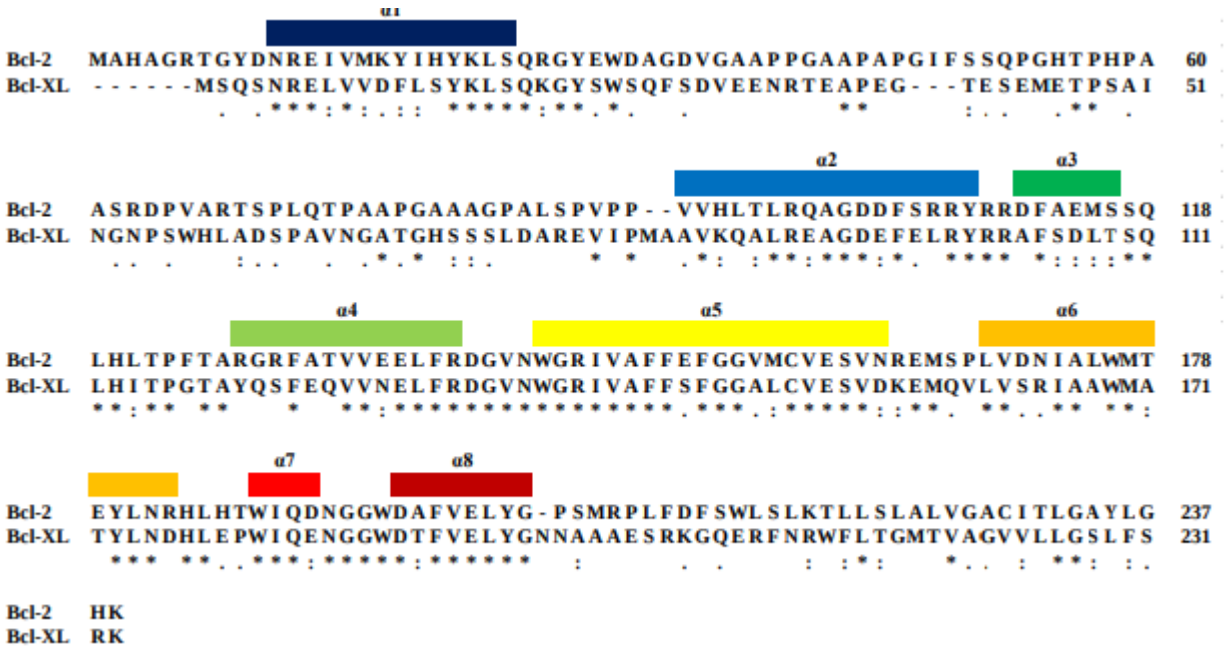

Figure 7: Sequence alignment of Bcl-2 and Bcl-XL generated with ClustalW. In the sequences, an asterisk (*) indicates an identical or conserved residue, a colon (:) indicates a conserved substitution, and a period (.) indicates a semiconserved substitution. Helices 1–8 of Bcl-2 and Bcl-XL are indicated with rectangular boxes above the sequences.