FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

MSc in Biochemistry
Dissertation Project – 2nd Cycle

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Lab/Institution: Macromolecular Crystallography Laboratory, UCIBIO, FCT-UNL
TITLE: Structure based drug design for the discovery of promising anticancer molecules

BACKGROUND

Apoptosis or Programmed Cell Death (PCD) is a vital process common to various biological mechanisms such as the proper functioning of the immune system and the embryonic development. This physiological process comprises a series of events, namely cell shrinkage, plasma membrane blebbing, chromatin condensation and nuclear fragmentation. Among the different proteins involved in the apoptotic process, the B-cell lymphoma2 (BCL-2) family of proteins play a vital role in the regulation of the PCD mechanism. This family of proteins can be broadly divided in two classes: those that inhibit the apoptotic process - Antiapoptotic, including the Bcl-2 protein, and those that promote apoptosis - Proapoptotic.
In many cancers, such as melanoma, breast, prostate or lung cancer, an enhancement or overexpression of Bcl-2 family of antiapoptotic proteins is observed, which increases their resistance to chemotherapeutics. Inhibition of these antiapoptotic members of the Bcl-2 family of proteins should target the abnormal apoptotic pathway in cancer cells, becoming a very attractive candidate drug target for therapy.

Identification of new promising molecules/potential inhibitors, increase the efficacy of the existing molecules or reduction of the side effects of currently used antiapoptotic proteins inhibitors are essential for the development of new therapeutics to treat various cancers.
Hence, in this project, we are going to use several biochemical and biophysical techniques to understand the structural features and the interaction mechanisms of the Bcl-2-inhibitor interaction in order to identify and design more efficient anticancer molecules.

OBJECTIVES

The aim of this work is to study the interaction between the human Bcl-2 protein and potential inhibitors of this protein. For this purpose, in this work we want to obtain the 3D structure of this protein in complex with potential inhibitors, already available in the laboratory. Saturation-Transfer Difference (STD) Nuclear Magnetic Resonance (NMR) and Isothermal Titration Calorimetry (ITC) experiments will be conducted in order to characterize protein-inhibitor interactions.

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PROJECT DESCRIPTION

In this project, several techniques will be used to characterize Bcl-2-inhibitor complex interactions.

Task 1 – **Protein overexpression and purification.** The human Bcl-2 protein has already been cloned in the laboratory and is going to be overexpressed in *Escherichia coli* and purified using affinity and size exclusion chromatography.

Task 2 – **ITC and STD-NMR experiments.** STD-NMR experiments are going to be performed to map the binding epitopes of the different ligands to human Bcl-2 protein. Binding affinities will be determined by ITC, which will allow the further characterization of the ligand binding and detect potential high affinity drug like molecules. The combination of both techniques will give structural and dynamic information regarding the protein-ligand interactions.

Task 3 – **X-ray Crystallography.** Protein crystals will be prepared and optimized. Soaking and co-crystallization experiments will be conducted to obtain protein-inhibitor complexes crystals.

Task 4 – **Data collection and processing.** X-ray diffraction experiments are going to be conducted using a home source and synchrotron radiation sources.

Task 5 - **Structure determination and analysis.** The three-dimensional structure of the human Bcl-2 protein will be determined using the models already available in the Protein Data Bank (PDB). This structure will then be used to determine the structure of protein-inhibitor complexes. Analysis of the protein-ligand complex will provide structural determinants for the understanding of the mode of action of the inhibitors and clues to design more efficient drug-like molecules.

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Task 5										
Thesis										