FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

MSc in Biochemistry
Dissertation Project – 2nd Cycle

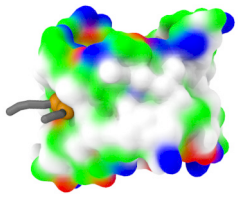
Student´s Name:
Student email address: No.
Supervisor(s): Manuel N. Melo
Supervisor(s) email address: m.n.melo@itqb.unl.pt
Lab/Institution: Multiscale Modeling Lab, MOSTMICRO — ITQB NOVA
TITLE: Simulating the oligomerization of apoptotic proteins in the outer mitochondrial membrane

BACKGROUND

The role of mitochondria in apoptosis is well established, and a number of protein–protein interactions in its outer membrane has been linked to apoptotic pathways. The VDAC (voltage-dependent anionic channels) family of proteins is central to this binding, having been implicated in the retention of the BAX protein in the outer mitochondrial membrane, which is a precursor step in apoptosis[1]. VDAC proteins are also known to oligomerize[2] — an event also linked to apoptosis — and to bind hexokinase-I, promoting increased metabolic rates and cell growth/tumor proliferation[3].

In a recent study we have ascertained that ceramide binds VDAC proteins and that such binding somehow triggers apoptosis[4]. An immediate hypothesis is that the presence of ceramide affects the binding profile of VDAC and promotes apoptosis-associated oligomerizations.

This project aims at simulating the partner preferences of outer mitochondrial membrane apoptotic proteins, in the presence and absence of ceramide, and correlating it with apoptosis.



Side view of ceramide bound to VDAC1, from [4].

1 – Sci. Rep. 6, 32994; doi: 10.1038/srep32994 (2016)
2 – Mol. Cell. Biol. 30, 5698; doi: 10.1128/MCB.00165-10 (2010)
3 – Biochim. Biophys. Acta 1848, 2547; doi: 10.1016/j.bbamem.2014.10.040 (2015)
4 – Nat. Commun. 10, 1832; doi: 10.1038/s41467-019-09654-4 (2019)

OBJECTIVES

– To employ coarse-grain modeling to simulate outer mitochondrial membrane proteins, namely VDACs and BAX.

– To determine the preferred dimerization organization of VDAC either as homodimer, or in conjunction with BAX.

– To gauge the effect of the presence of ceramide in the dimerization preferences.

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PROJECT DESCRIPTION

As with our recently-published work on VDAC–ceramide binding, this project will employ coarse-grained simulations. These simplified molecular representations allow the tackling of the size and timescales required for this project.

Four main tasks will be followed:

Task 1 – To simulate VDAC as a dimer and to observe preferred binding modes. This, and the subsequent tasks, will save work by making use of the protein and membrane models already developed for the VDAC–ceramide study.

Task 2 – Given the likelihood that the system modeled in Task 1 may get stuck in less relevant energy minima, an advanced simulation method will be employed where all the binding interfaces are tested.

Task 3 – To extend the simulation strategy to probe the VDAC–BAX binding.

Task 4 – To reassess all binding energies in the presence of ceramide.

The student will be trained in an array of computing techniques with wide applicability beyond the scope of the project and even outside academia. These include the use of simulation software, structural/dynamic data analysis methods, and overall experience with open-source operating systems.

TIMELINE

	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										
Thesis										

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Supervisor(s) email address: m.n.melo@itqb.unl.pt
Lab/Institution: Multiscale Modeling Lab, MOSTMICRO — ITQB NOVA

TITLE: Characterizing the quinone binding sites in *S. aureus* respiratory oxireductases by coarse-grained simulations

BACKGROUND

Even though the bacterial respiratory chain is closely analogous to the eukaryotic one, differences do exist and make for plausible targets for antimicrobial therapies. To that end, it becomes useful to understand the catalysis mechanism. Coarse-grain (CG) simulations have recently emerged as a powerful technique to study such enzyme–substrate interactions in a respiratory context, providing unrivaled structural and dynamic detail[1].

Several oxidoreductases specific to *Staphylococcus aureus* have been identified and demonstrated to interact with quinone/quinol molecules[2]. *S. aureus* is an opportunistic pathogen and one of the most frequent causes for community acquired and nosocomial infections. It has become a major public health threat due to the increased incidence of its drug resistance. Preliminary CG simulations in a representative bacterial membrane have been able to identify putative binding sites for some of these oxidoreductases. This opens the door to a number of simulations to characterize the catalysis and to devise modifications that can affect efficiency.

1 – Nat. Commun. 8, 15214 doi: 10.1038/ncomms15214 (2017)
2 – Sci. Rep. 7, 42303; doi: 10.1038/srep42303 (2017)

OBJECTIVES

The goals of this project are to characterize quinone reductase catalysis from CG simulations:

– To identify and characterize further quinone/quinol binding sites;

– To quantify quinone binding rates;

– To compare the behavior of the quinol products;

– To identify and test mutation targets to modulate activity.

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PROJECT DESCRIPTION

The project is divided into four tasks.

Task 1 – To set up multiple simulations of quinone–enzyme binding, for different oxireductases. This includes simulations in aqueous environment, for soluble oxireductases, and in a membrane environment, for membrane-bound oxireductases;

Task 2 – Starting from states of bound quinones obtained in Task 1, to modify them to quinols and follow their unbinding dynamics;

Task 3 – For both Task 1 and Task 2 analyze times to binding/unbinding and identify binding site access/egress pathways;

Task 4 – From the information in Task 3 infer common properties of quinone/quinol binding proteins. These hypothetical determinants of quinone/quinol binding can then be tested by simulating the impact of their mutation in activity, as data for future experimental validation.

The student will be trained in an array of computing techniques with wide applicability beyond the scope of the project and even outside academia. These include the use of simulation software, structural/dynamic data analysis methods, and overall experience with open-source operating systems.

TIMELINE (use fill tool for the cells)

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Supervisor(s) email address: m.n.melo@itqb.unl.pt
Lab/Institution: Multiscale Modeling Lab, MOSTMICRO — ITQB NOVA

TITLE: Poking holes in membranes with coarse-grain simulations

BACKGROUND

Membrane poration and membrane fusion are central events in several different biological processes. As examples, formation of pores in bacterial membranes is one of the proposed mechanisms of the antimicrobial peptide (AMP) class of antibiotics, and membrane fusion is a crucial step in the infection by enveloped viruses. Through molecular-level simulation of these processes one can hope to understand the factors responsible for poration/fusion and develop strategies to enhance the behavior (in the case of the AMPs), or to inhibit it (in the case of enveloped viral infection).

The Martini coarse-grain molecular dynamics model can faithfully reproduce a number of cell membrane properties, having allowed the observation of very relevant biological processes. The size and timescales involved in pore formation and membrane fusion are within the reach of the model. However, these phenomena are typically poorly represented by Martini, and any improvement at this level is sure to have strong impact and applicability.

OBJECTIVES

This project focuses on identifying the limitations involved in pore simulations with the Martini model and from there to propose improvements to more accurately model these.

If a successful model is developed, it can then be applied to simulating AMP action, in an attempt to observe spontaneous pore formation.

Given the similarities between the events of membrane poration and of membrane fusion, a successful outcome lays the foundation for further fusion-centered work.

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PROJECT DESCRIPTION

The strategy of model diagnosis–improvement–application strate can be divided into three main tasks:

Task 1 – To employ advanced modeling techniques to force a system into porated states and visualizing elements that oppose this, in terms of free-energies. For reference, values will be compared to full-detail simulation results.

Task 2 – To adjust elements of the coarse-grain model to correct for the discrepancies identified in Task 1. To iteratively repeat tasks 1 and 2 until a better agreement with finer models is achieved.

Task 3 – To apply the improved model to AMP simulations, towards the pioneering simulation of spontaneous pore formation.

Expected outcomes are models that allow unbiased simulation of pore formation, and, by extension the design of conditions that can promote pore-forming behavior. These can ultimately be translated into therapeutic strategies.

The student will be trained in an array of computing techniques with wide applicability beyond the scope of the project and even outside academia. These include the use of simulation software, structural/dynamic data analysis methods, and overall experience with open-source operating systems.

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Student email address: No.
Supervisor(s): Manuel N. Melo, Federico Herrera
Supervisor(s) email address: m.n.melo@itqb.unl.pt, fherrera@itqb.unl.pt
Lab: Multiscale Modeling Lab, Cell Structure and Dynamics Lab / MOSTMICRO — ITQB NOVA
TITLE: **Chopping and re-joining: predicting how protein fragments combine to rescue function**

BACKGROUND

Protein complementation (PC) is the process by which two fragments of a protein combine to recover part of the full protein's function. This property has been extensively used with fragments of fluorescent proteins in reporting interactions between other proteins (the so-called bi-functional complementation)[1]: when two target proteins A and B are chimerically expressed together with fragments of a fluorescent protein, complementation will cause the ensemble to fluoresce whenever A and B interact and bring the attached fragments together.

Because of this main application most of the research into PC has focused on fluorescent proteins and their fragments' complementation when in constructs with other proteins. This project, however, focuses on PC as an independent process and will try to predict how proteins can be chopped in order to yield fragments that will successfully complement on their own. Being able to predict PC is in itself a valuable biotechnological tool, but it also has an immediate application in diseases caused by protein truncation: one will be able to design complementing fragments that rescue the healthy function of the affected protein.

This multidisciplinary project will employ machine-learning techniques on several aspects of available PC data to create predictive models of PC on different types of proteins. The modeling results will then be used to instruct experimental validation and guide the experimental extraction of further features.

1 – Biotechniques 2012, 52: 45-50

OBJECTIVES

– To build and train a model on the available data on PC that can predict when a given protein fragmentation will be able to complement and regain function;

– To propose and test experimental validation fragmentations, both of predicted active and inactive PC fragment pairs;

– To apply the model to real-world situations of protein truncation involved in disease.

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PROJECT DESCRIPTION

This is a multidisciplinary project divisible into 5 logical tasks:

Task 1 – To analyze available experimental data on PC — tests of different cuts of different proteins — and identify candidates for feature extraction.

Task 2 – To extract a number of features from candidate PC cases in Task 1. This project aims at being a non-hypothesis-driven approach, and as such it will include as many features from each PC case as possible. Nonetheless, since it can be expected that PC will depend to a degree on structural characteristics of each fragment, emphasis will be on structural features when available and on characterizing the interface of fragmentation (its size and polar character).

Task 3 – To use the collected variables in the building and cross-validation of a predictive model for the success of a given protein and fragmentation points, taking care to employ a machine-learning model type appropriate to the expected small number of data points.

Task 4 – To experimentally validate the proposed model by creating predicted-positive and predicted-negative fragment combinations of a given protein. The outcome of this task will further guide model development.

Task 5 – To run the model on different identified cases of disease by protein truncation. To highlight cases for future exploration for which the model predicts complementary fragments that can rescue function.

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