

MSc Dissertation Project – 2nd Cycle

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Scientific area: Nano-biotecnologia

TITLE: Nano - biotechnology applied to protein crystallization: The effect of nanoparticles-induced crystallization of enzymes in ionic liquid media with and without presence of functionalized ligands

BACKGROUND

In the modern structural biology field, there is a concern in obtaining a good X-ray diffracting protein crystal without changing the function of the protein. For this, we are in the situation to introduce some additives such as ions, salt, and nanoparticles, etc., for increasing the stability and solubility of the protein. Ionic liquids have been found to be more effective as additives in protein crystallization, with different ionic liquids used to increase crystallization rates and crystal size with less crystal polymorphism as well as less precipitation at higher precipitant concentrations. In this project, several enzymes (trypsin, RNase A and Proteinase K) are going to be crystallized with different ionic liquid media with and without different functionalized ligands including different nanoparticles. The catalytic activity and thermo stability of the enzyme will be tested with and without the presence of those additives to characterize the function of the enzyme. In the future, this project will help developing the ionic liquid media for getting crystals of enzymes with the presence of nanoparticles with the aim to increase the thermo stability of the enzyme, which is useful for industrial purpose.

OBJECTIVES

Aim of this project divided in to three major parts.

1. Three enzymes (trypsin, RNase A and Proteinase K) are going to be co-crystallized with ionic liquid media in various concentrations of ionic liquid with and without presence of different functionalized ligands.
2. All the enzymes will be co-crystallized with different nanoparticles in various concentrations of nanoparticles.
3. To study the catalytic activity and thermo stability of the enzyme with and without the presence of nanoparticles in ionic liquid media.

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PROJECT DESCRIPTION

This project includes the synthesis of nanoparticles, preparation of protein and different ionic liquid media for the enzyme crystallization. The crystallization of enzymes will be carried out using known crystallization conditions proposed by previous literatures. The student will be involved in the crystallization experiments using hanging drop vapour diffusion methods. Also, the student will be trained in x-ray data collection using the home source x-ray machine, solving the structure of proteins using different programs and validation of refined protein structure followed by interpretation across the three dimensional structure in the electron density maps. The student will also be involved in enzymatic assay experiment to analyse the catalytic activity of the enzyme with and without the presence of nanoparticles in ionic liquid media.

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 | Optimization of crystallization condition with different ionic liquid media with and without nanoparticles | | | | | | | | | |
| Task 2 | | X-ray diffraction data collection | | | | | | | | |
| Task 3 | | | | 3D structure determination and resolution analysis | | | | | | |
| Task 4 | | | | | Enzymatic assay analysis | | | | | |
| Task 5 | | | | | | | | | | |
| Task 6 | | | | | | | | | | |
| Thesis | | | | | | | | Thesis writing | | |