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Lab/Institution: Dynamic Structural Biology Lab / MOSTMICRO — ITQB NOVA
TITLE: **Host-Pathogens interactions blocking immune response**

BACKGROUND

Innate immunity is the first line of defence against pathogens such as viruses and bacteria. Nevertheless, pathogens have evolved mechanisms to evade immune response during infection, often by delivering a cocktail of effectors that mimic the activity of host proteins, as a sophisticated strategy to turn host functionality to their advantage. The effector Tir secreted by EHEC/EPEC and the oncogenic virulence factor CagA from *H. pylori*, share sequence similarities with the immunoreceptor tyrosine-based inhibition motifs (ITIMs), often found in the cytoplasmic tails of inhibitory receptors of the immune system. After phosphorylation of those ITIM motifs by host kinases, Tir directly associates with tyrosine phosphate SHP1 and nucleates a Tir-SHP1-TRAF6 complex to suppresses immune responses.

On this topic, we are looking for a Master student eager to understand at the atomic level how pathogens evade immune defence. Within this project, the candidate will employ hybrid structural biology approaches to gain unprecedented insights into the mechanisms of **how Tir binds to and aberrantly recruits** SHP1 to a complex with TRAF6**, and in turn, disarm host-defence mechanisms.**

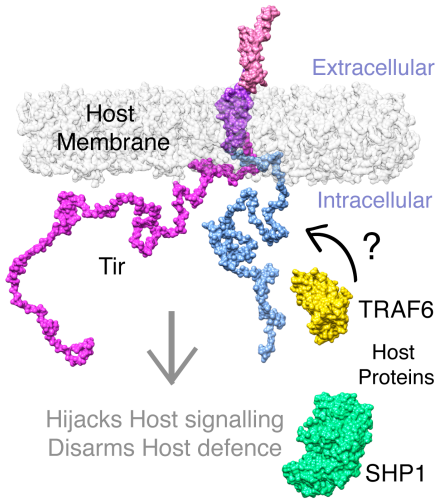

Fig.1 – Schematic of Tir recruiting SHP1 to a complex with TRAF6 to suppress immune response

OBJECTIVES

The goals of this project are to unveil the structural determinants underpinning Tir-SHP1 interaction

This consists of several sub-goals:

– To assess the differential binding of Tir ITIM-like motifs to SHP1

– To probe the effect of Tir on SHP1 enzymatic activity.

– Ultimately, provide dynamic structural information on Tir-SHP1 proteins interactions.

PROJECT DESCRIPTION

The project is divided into 4 highly interconnected tasks, roughly divisible along the sub-goal lines described above.

Task 1 – Protein expression and purification. **Clones for most Tir and host proteins constructs are available, and overexpression and purification have been tested and optimized,** so that large-scale production, including isotope labelling for NMR experiments, can be done using *in-house* facilities. Exploiting directly from preliminary results, the project will involve expression and purification of the host SHP1 protein.

Task 2 – Binding strength of Tir and peptides to SHP1 SH2 domains. Tir has 3 ITIM-like motifs within its C-terminal region. To dissect the Tir-SHP1 interaction, we will quantitatively determine the binding affinities of tyrosine-phosphorylated Tir-peptides and constructs with individual N-SH2 or C-SH2, and tandem SH2-domains of SHP1.

Task 3 – SHP2 Activation by Tir. Next, we will investigate the effect of Tir on the catalytic activity of SHP1 by an *in vitro* phosphatase assay using p-nitrophenylphosphate as a substrate. The student will probe the contribution of individual Tir-motifs to Tir-mediated SHP1 activation.

Task 4 - Solution structure Analysis of Tir-SHP1 complex. The applicant will use advanced NMR tools and SAXS to leverage the existing crystal structures of SHP1 and build an atomic-resolution structure of the Tir-SHP1 complex. Nevertheless, we expect to crystallize the globular partners in the presence of a small peptides corresponding to interacting regions of *Tir (collaboration).*

Task 4.1 - ***S*ynchrotron SAXS will be performed at Large European Facilities** to investigate the conformational change of SHP1 upon binding Tir. DSB lab is a regular user of such international facilities with a strong track record for obtaining the required beam-time.

Task 4.2-To further elucidate the structural basis underlying the Ti-Host interaction we will collect high-NMR data at the the highest field NMR-facilities in Portugal (CERMAX) host by ITQB. To this aim, isotopically (15N/13C) labelled constructions of Tir will be overexpressed in *E. coli* and subsequently purified using standard procedures. *In-house* NMR resources will be applied to assign sequence-specific NMR resonances and capture protein structure and dynamics at high precision upon binding.

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 4.1										
Task 4.2										
Thesis										