
# Concursos de Projectos de I&D
Calls for R&D Projects

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## Visão global da candidatura
Application overview

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**Referência do projecto**
Project reference
PTDC/SAU-MIC/117060/2010 **(Lacrado a 23-02-2011 às 15:57)**

## 1. Identificação do projecto -
1. Project description

**Área científica principal**
Main Area
Ciências da Saúde - Microbiologia e Infecção

**Área científica Secundária**
Secondary area
Ciências Biológicas - Biologia Microbiana

**Título do projecto (em português)**
Project title (in portuguese)
Utilização dos recursos da célula hospedeira pelo parasita da malária no fígado

**Título do projecto (em inglês)**
Project title (in english)
Utilization of host cell resources by the malaria parasite in the liver

**Financiamento solicitado**
Requested funding
197.566,00€

**Palavra-chave 1**
Plasmodium

**Palavra-chave 2**
Interacções parasita-hospedeiro

**Palavra-chave 3**
Homeostase

**Palavra-chave 4**
Sinalização

**Keyword 1**
Plasmodium

**Keyword 2**
Host-parasite interactions

**Keyword 3**
Homeostasis

**Keyword 4**
Signaling

**Data de início do projecto**
Starting date
01-01-2012

**Duração do projecto em meses**
Duration in months
36

## 2. Instituições envolvidas
2. Institutions and their roles

-

**Instituição Proponente**
Principal Contractor

**Instituto de Medicina Molecular (IMM/FM/UL)**
Avenida Professor Egas Moniz
1649-028Lisboa

**Instituição Participante**
Participating Institution
*(Vazio)*
*(Void)*

**Unidade de Investigação**
Research Unit

**Instituto de Medicina Molecular (IMM/FM/UL)**
Avenida Professor Egas Moniz
1649-028Lisboa

**Unidade de Investigação Adicional**
Additional Research Unit
*(Vazio)*
*(Void)*

**Instituição de Acolhimento**
Host Institution
*(Vazio)*
*(Void)*

## 3. Componente Científica
3. Scientific Component

-

### 3.1. Sumário
**3.1 Abstract**

**3.1.a Em português**
3.1.a In Portuguese

A malária é uma doença devastadora. Em tempos considerada perto da erradicação, hoje em dia é responsável por praticamente meio bilião de infecções e um milhão de mortes anuais. A primeira fase da infecção de mamíferos pelo Plasmodium, o agente causador da malária, ocorre no fígado do hospedeiro. Embora seja clinicamente silenciosa, esta fase da infecção é obrigatória e é responsável por um aumento no número de parasitas de pelos menos duas ordens de magnitude. A sua natureza assintomática torna a fase hepática da infecção por Plasmodium um alvo ideal para uma intervenção profilática. Contudo, esta é também a fase menos estudada e compreendida do ciclo de vida do parasita, e aquela onde importantes questões permanecem por responder.
O nosso laboratório tem estado na linha da frente da investigação da fase hepática do Plasmodium, tendo dado importantes contribuições para aumentar a nossa compreensão acerca das interacções que ocorrem na interface parasita-hospedeiro. Revelámos aspectos cruciais da infecção de hepatócitos pelo Plasmodium, desenvolvemos metodologias para estudar a infecção de células do fígado pelo parasita e identificámos factores chave do hospedeiro que influenciam o seu desenvolvimento. Neste projecto, propomo-nos desvendar aspectos até agora desconhecidos da infecção do fígado por Plasmodium, as quais podem ser resumidas pela questão: como é que o Plasmodium subverte a sua célula hospedeira de modo a obter os nutrientes necessários para o seu desenvolvimento intra-hepático, a regular a homeostase da célula hospedeira e a assegurar a sobrevivência desta?
Propomo-nos responder a esta questão baseados em diversas observações publicadas pelo nosso laboratório em conjunto com uma série de experiências preliminares sólidas. Em concreto, mostrámos que (i) transportadores de membrana da célula hospedeira são modulados durante o desenvolvimento do Plasmodium e parasitas intracelulares são capazes de activar a actividade de canais iónicos na membrana citoplasmática da célula hospedeira; (ii) o transportador de glucose do Plasmodium é essencial para o desenvolvimento dos estados hepáticos do parasita e a glucose favorece o desenvolvimento intrahepático do Plasmodium conduzindo simultaneamente a um aumento da sobrevivência das células hospedeiras infectadas; (iii) vários ligandos, agonistas e antagonistas de receptores nucleares (NR) influenciam de forma marcada o desenvolvimento do Plasmodium dentro de células de hepatoma humano e diminuição da expressão de diversos NR por RNA de interferência influencia a infecção de células do fígado por Plasmodium. Estas observações conduziram a três hipóteses que agora nos propomos abordar: (I) O Plasmodium usa transportadores específicos do hospedeiro para regular o equilíbrio iónico, manter a homeostase e preencher as suas necessidades nutricionais; (II) O Plasmodium modula a aquisição de glucose pela célula hospedeira de modo a desenvolver-se e assegurar a sobrevivência da célula hospedeira; e

(III) Vias de sinalização mediadas por receptores nucleares regulam a homeostase enquanto asseguram um fornecimento contínuo de nutrientes ao parasita.
Para testar estas hipóteses, utilizaremos a nossa experiência e conhecimento em conjunto com os dos nossos colaboradores e consultores neste Project. Faremos uso de metodologias de ponta, incluindo microscopia confocal de imunoflurescência, RNA de interferência e PCR quantitativo em tempo real. Estas técnicas serão aplicadas a modelos de infecção in vitro já estabelecidos bem como a modelos roedores de malária, em relação aos quais o nosso laboratório tem uma vasta experiência. Isto permitir-nos-á abordar experimentalmente aspectos fundamentais do transporte de solutos e iões através da membrana de células infectadas, aquisição de energia pelo parasita, controle da homeostase e regulação da sobrevivência da célula hospedeira.
Esperamos que os nossos estudos revelem aspectos fundamentais da infecção do fígado pelo Plasmodium e da sua interacção com a célula hospedeira. Para além disso, contamos identificar moléculas e vias do hospedeiro que possam ser exploradas enquanto alvos de intervenção contra a malária. O potencial dos alvos assim identificados será explorado e poderá conduzir a novas estratégias profiláticas. Esta preocupação é particularmente actual dado o facto da fase hepática da infecção ser um alvo ideal de intervenções profiláticas mas também aquele contra o qual existe uma óbvia carência de compostos eficazes.

### 3.1.b Em inglês

3.1.b In English

Malaria is a devastating disease. Once thought to be close to eradication, it is now responsible for nearly half a billion infections and one million deaths every year. The first stage of mammalian infection by Plasmodium, the causative agent of malaria, occurs in the host's liver. Although clinically silent, this phase of infection is obligatory and is responsible for an increase in parasite numbers of at least two orders of magnitude. Its asymptomatic nature makes the liver stage of Plasmodium infection an ideal target for prophylactic intervention. However, this is arguably the least studied and understood stage of the parasite's life cycle and one where several important questions remain unanswered.
The host laboratory has been at the forefront of Plasmodium liver stage research, having made significant contributions to further the understanding of the interactions that take place at the host-parasite interface. We have unveiled crucial aspects of hepatocyte infection by Plasmodium, developed techniques to study the parasite's infection of liver cells and identified key host factors that influence its outcome. In the present project we propose to unveil hitherto unknown aspects of Plasmodium liver infection, which can be summarized by the question: how does Plasmodium subvert its host to gain access to the essential nutrients it requires for intra-hepatic development, to regulate homeostasis of its host cell, and to ensure survival of the host cell?
We set out to answer this question based on several published observations from our laboratory alongside an array of robust preliminary results. Specifically, we have shown that (i) host membrane transporters are modulated during Plasmodium development and intracellular parasites activate volume-regulated anion channel-like activity in the host cell plasma membrane; (ii) the plasmodial glucose transporter is essential for development of Plasmodium liver stages and glucose enhances Plasmodium intra-hepatic development while leading to increased survival of infected host cells; (iii) a number of nuclear receptor (NR) ligands and synthetic NR agonists or antagonists markedly influence development of Plasmodium in human hepatoma cells and RNAi-mediated down-modulation of several NRs impairs infection of liver cells by Plasmodium. These observations raised three hypotheses that we now propose to address: (I) Plasmodium uses specific host transport proteins to regulate ionic balance, maintain homeostasis and fulfill its nutritional needs; (II) Plasmodium modulates uptake of glucose by hepatocytes in order to develop and to ensure host cell survival; and (III) Nuclear receptor signaling pathways regulate homeostasis while ensuring a continuous supply of nutrients to the parasite.
In order to test these hypotheses, we will use our own expertise alongside that of our collaborators and consultants in this project. We will employ state-of-the-art methodologies including immunofluorescence confocal microscopy, RNA interference, quantitative real-time PCR, and patch-clamping. These will be used in combination with established in vitro models of infection as well as rodent models of malaria, both of which the host laboratory has a vast experience with. This will enable us to address experimentally key aspects of solute and ion transport across infected cell membranes, energy acquisition by the parasite, control of homeostasis, and regulation of cell survival.
We expect that our studies will reveal fundamental aspects of liver infection by Plasmodium and its interaction with its host cell. Furthermore, we expect to identify host molecules and pathways that can be exploited as anti-malarial targets. The potential of identified druggable host factors will be investigated and may lead to novel prophylactic strategies against malaria. This is a particularly timely concern given that the liver stage of infection is an ideal target for prophylactic intervention and one for which there is an obvious shortage of effective compounds.

## 3.2. Descrição Técnica

**3.2 Technical Description**

### 3.2.1. Revisão da Literatura

3.2.1. Literature Review

Malaria is caused by Plasmodium parasites injected in their mammalian host through the bite of an infected Anopheles mosquito. In order to reach the bloodstream in a form capable of causing disease symptoms, Plasmodium parasites first undergo an obligatory and unidirectional developmental phase in the liver (1). The host laboratory has been at the forefront of hepatic stage infection research, identifying mammalian host factors that influence Plasmodium liver infection (2-5). It is now widely accepted that host factors are crucial determinants of the outcome of Plasmodium liver infection (6, 7, Fig.1).
A microarray study from our lab showed that Plasmodium development inside the hepatocyte induces the coordinated and sequential expression of host genes (8). Prominent among these are genes involved in the uptake of exogenous metabolites, namely genes encoding membrane transporters (MTs). MTs are required to obtain nutrients, remove toxic metabolites, and regulate cell volume. Our analysis revealed 66 genes coding for MT proteins or regulatory subunits that are differentially expressed throughout infection, including several ion channels, amino acid transporters, P-type ATPases and ABC transporters (8). Most strikingly, we observed that i) genes involved in amino acid transport are up-regulated throughout infection and ii) pathways directly associated with regulatory

volume increase and decrease are up- and down-regulated during infection, respectively (8). Recently, we also showed that the intracellular parasite activates volume-regulated anion channel-like activity in the host cell plasma membrane (9). These studies suggest that intra-hepatic Plasmodium parasites modulate host's MTs, most likely to ensure their own survival and development. Recently, we also recently showed that glucose plays a crucial role during the liver stage of P. berghei, establishing the importance of glucose import through the parasite's membrane for its replication inside liver cells (10). In view of this, we postulated that the uptake of glucose by the host cell would likely be a crucial requirement for the parasite's ability to develop normally. Thus, we carried out preliminary experiments to assess whether and how the availability of glucose would influence Plasmodium infection of hepatic cells. Our results showed that (i) the parasite load of infected liver cells correlates with the amount of available glucose in the medium (Fig. 2-A); (ii) glucose levels positively correlate with the extent of Plasmodium's intracellular replication (Fig. 2-B) and (iii) increased glucose availability leads to enhanced survival of infected cells (Fig. 2-B). Overall, these data demonstrate that the parasite's development inside liver is intimately linked to the availability of glucose to the host cells, suggesting that glucose uptake by hepatocytes is a crucial determinant of the fitness of Plasmodium liver stages. Glucose a fundamental source of energy and thus constitutes a crucial nutrient for cells. It is transported into hepatocytes via several isoforms of the facilitative glucose transporter (GLUT). In the adult liver, GLUT-2 is the primary expressed glucose bidirectional transporter and has been linked to HCV infection of hepatoma cells and hepatocytes (11, 12). GLUT1, GLUT3 and GLUT9 have been identified as the major contributors to glucose influx in HepG2 cells, a human hepatoma cell line (13). GLUT 4 is responsible for insulin-stimulated glucose uptake in several cell-types and tissues, including hepatocytes (14).

The parasite-induced increase in metabolite concentration inside the host cell is likely to activate regulatory pathways aimed at maintaining homeostasis. Nuclear receptors (NRs) are ligand-activated transcription factors that tightly control numerous cellular processes, including the transport of ions and molecules across membranes and metabolic pathways (15). NRs have also been shown to influence glucose metabolism by regulating gluconeogenesis, glycogenesis and incretin release (16). In response to changing levels of their ligands, NRs drive compensatory changes in gene expression to maintain homeostasis (17). Interestingly, the liver is the organ that produces or collects most known NR ligands, such as various derivatives of cholesterol. We assessed the effect of various NR ligands on Plasmodium liver stage infection in vitro and showed that several NR ligands, agonists and antagonists markedly influence the development of P. berghei in human hepatoma cells (Fig.3). Thus, we postulated that NR-mediated signaling might interfere with ion and metabolite homoeostasis and thereby influence Plasmodium infection of liver cells. To test this, we have used RNAi to screen a library of the 49 known NRs and monitor the effect of their knock-down on infection of hepatoma cells by Plasmodium. Our preliminary results showed that down-modulation of several NR leads to marked effects on infection (Fig. 4). These results indicate that interfering with NR-mediated regulation and, therefore, with the metabolic processes they control, clearly influences the development of Plasmodium.

In view of these observations, we now propose to investigate the following hypotheses: (I) Plasmodium uses specific host transport proteins to regulate ionic balance, maintain homeostasis and fulfill its nutritional needs; (II) Plasmodium modulates uptake of glucose by hepatocytes in order to develop and to ensure host cell survival; and (III) Nuclear receptor signaling pathways regulate homeostasis while ensuring a continuous supply of nutrients to the parasite. The results obtained will further our understanding of Plasmodium's nutrient requirements during the liver stage of infection and of the mechanisms it employs to subvert host cell's resources and ensure its survival and developmental needs. Importantly, we expect that this knowledge will identify novel potential drug targets and new strategies for anti-malarial intervention.

### 3.2.2. Plano e Métodos

3.2.2. Plan and Methods

In this project, we propose to fill important gaps in our understanding of the obligatory liver stage of infection by Plasmodium, the causative agent of malaria. In view of the established notion that host factors play a crucial role during this stage of the parasite's life cycle, we now aim to investigate the following hypotheses (Fig.1):

(I) Plasmodium uses specific host transport proteins to regulate ionic balance, maintain homeostasis and fulfill its nutritional needs;
(II) Plasmodium modulates uptake of glucose by hepatocytes in order to develop and to ensure host cell survival;
(III) Nuclear receptor signaling pathways regulate homeostasis while ensuring a continuous supply of nutrients to the parasite.

These hypotheses stem from an array of recently published observations from our lab and from a set of robust preliminary data, as described in detail in the "Literature Review" section of the present proposal (Fig. 1). Briefly, we have shown that (i) host membrane transporters are modulated during Plasmodium development (8) and intracellular parasites activate volume-regulated anion channel-like activity in the host cell plasma membrane (9); (ii) the plasmodial glucose transporter is essential for development of Plasmodium liver stages (10) and glucose enhances Plasmodium intra-hepatic development while leading to increased survival of infected host cells (Fig. 2); (iii) several NR ligands and synthetic NR agonists or antagonists markedly influence development of Plasmodium in human hepatoma cells (Fig.3) and RNAi-mediated down-modulation of several nuclear receptors impairs infection of liver cells by Plasmodium (Fig.4).

The hypotheses outlined above will be interrogated during six tasks, where we will investigate:

Task #1- Nutrient uptake by host membrane transporters during liver infection by Plasmodium
Task #2- Ion transport and cell homeostasis in Plasmodium-infected liver cells
Task # 3- Glucose uptake by Plasmodium-infected cells and glucose-dependent parasite development
Task #4- The role of glucose on survival of Plasmodium-infected cells
Task #5- Regulation of homeostasis and nutrient supply to Plasmodium by host nuclear receptors
Task #6- Assessment and validation of anti-malarial strategies

These tasks were carefully designed to ensure that they can be carried out independently from one another. This is to avoid the situation in which the execution of one task is precluded by delays or unexpected results from another. Nevertheless, our hypotheses concern different aspects of a single fundamental question, that of how Plasmodium subverts its host to gain access to the essential nutrients it requires for intra-hepatic development, to regulate homeostasis of its host cell, and to ensure survival of the host cell. In that sense, results obtained in these tasks will not be treated independently. On the contrary, they will be analysed in the context of the overall process we are trying to elucidate and, whenever relevant, they will serve to feed other tasks and to inform new strategies

that may become relevant as the project progresses. Overall, we expect that the results obtained will be closely knitted to shed light on fundamental aspects of liver infection by Plasmodium. It should also be noted that besides this “basic biology” objective, there also exists a more “applied” perspective to this project, as we expect to identify host molecules and pathways that can be exploited as anti-malarial targets. Thus, we will investigate the potential of identified host factors as druggable targets that can be used as part of novel prophylactic strategies against malaria. This is particularly relevant if we consider that the liver stage of infection is an ideal target for prophylactic intervention and one for which there is an obvious shortage of effective compounds (1).
Completion of the proposed tasks will engage the expertise of the team members and consultants in areas such as cell biology, molecular biology, and membrane transport, and will make use of available resources such as high resolution fluorescence microscopy, quantitative real-time RT-PCR, patch-clamping, and animal models of malaria infection.

RNAi screens and in vitro infection readout
The selected genes of interest will be targeted by short interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs) in order to down-modulate their expression. We will employ an established in vitro infection model using Huh7 or HepG2 human hepatoma cells infected with P. berghei parasites, in 96-well plates. Parasites will be obtained from the dissection of the salivary glands of infected female Anopheles mosquitoes, routinely bred in the host laboratory (18).
The infection load of Huh7 cells by Plasmodium will be assessed by fluorescence (18) and luminescence (19) based methods that have been optimized in the host laboratory alongside established immunofluorescence microscopy techniques. Based on our previous work, SR-BI will be used as positive control in RNAi experiments (2, 3). Screening results will be integrated through bioinformatics analysis that will identify the most relevant host pathways involved in Plasmodium development.

Immuno-fluorescence microscopy and live imaging
For immunofluorescence microscopy, cells seeded on glass coverslips will be fixed and permeabilized as described before (3). Observations will be carried out on a Zeiss LSM Meta scanning confocal microscopes. For live confocal microscopy, Huh7 cells will be seeded on glass-bottomed imaging dishes and infected with GFP- or RFP-expressing P. berghei. Glucose pulse-chase experiments will be carried out on an Andor Revolution spinning-disk confocal system equipped with temperature and CO2 control. For FRAP measurements, fluorescent glucose will be bleached once and the fluorescence recovery inside that same region will be imaged using the Zeiss LSM system equipped with a chamber for temperature and CO2 control.

Cell-based reporter assay
Huh7 cells will be transiently co-transfected with i) a plasmid expressing the cDNA of the candidate nuclear receptor, and ii) a plasmid expressing a luciferase reporter gene under the control of a promotor specific of the given nuclear receptor (20). Infected cells will be monitored for NR-dependent luciferase activity.

Functional membrane transport assays
A range of functional transport assays that are amenable to single cells studies will be used. Patch-clamp methodologies, developed to study Plasmodium-infected liver cells (9) will enable the study of ion channels and electrogenic transporters. Alternatively, X-ray microanalysis will be used to study elemental concentrations in conjunction with electron microscopy, as used to study single Plasmodium-infected erythrocytes (e.g. 21) Fluorescence microscopy will be used to monitor solute transport, using labeled solutes such as 2-NBD-glucose.

Animal models
In vivo infection studies will employ C57BL/6 mice housed in the pathogen-free facilities of the Instituto de Medicina Molecular (IMM). Transgenic mice will be purchased from Jackson Laboratories. Drugs will be administered by intravenous or intraperitoenal injection, or by oral gavage. In vivo RNAi will be performed as previously described (2-4). Remaining specific gene-mRNA levels will be determined by qRT-PCR. Parasite liver loads will be determined by luminescence measurement following intra-dermal injection of luciferin (19). Blood stage infections will be followed by daily monitoring of parasitemia (% infected red blood cells) and disease symptoms. All protocols to be used are approved by the IMM Animal Care Committee.

The Malaria Unit (UMA) of the IMM, led by Prof. Maria M. Mota, a consultant in this project, has world-renowned expertise in malaria and, in particular, in the study of host factors that influence infection by Plasmodium. The PI is a Staff Scientist at the IMM-UMA and a liver-stage malaria expert who has written various reviews on this subject and has participated in numerous studies, published in international peer-reviewed journals. He has developed techniques for studying hepatic malaria infection and identifying host molecules that modulate it. Dr. G. Cabal and Dr. E. Real are experts in cell biology fluorescence microscopy and microbial infections. Dr. Henry Staines, a consultant in this project and a collaborator of the host laboratory, is a specialist in functional membrane transport measurement, with 15 years experience of studying Plasmodium-infected erythrocytes.

### 3.2.3. Tarefas
**3.2.3. Tasks**

**Lista de tarefas** (6)
**Task list** (6)

| **Designação da tarefa**<br>Task denomination | **Data de início**<br>Start date | **Data de fim**<br>End date | **Duração**<br>Duration | **Pessoas * mês**<br>Person * months |
|---|---|---|---|---|
| Nutrient uptake by host membrane tran... | 01-01-2012 | 31-12-2013 | 24 | 21,4 |

**Descrição da tarefa e Resultados Esperados**
Task description and Expected results

During their development inside hepatocytes Plasmodium parasites undergo major transformations and initiate a period of remarkable replication that requires the availability of significant amounts of nutrient and structural molecules (1). We have previously shown that Plasmodium development in liver cells is accompanied by changes in the expression of host genes throughout infection (8). Among these genes, 66 code for membrane transport (MT) proteins or regulatory subunits (8). It is particularly noteworthy that differentially expressed (DE) genes involved in amino acid transport are up-regulated throughout infection, warranting their prioritization in this study. However, the fact that a given gene is DE does not explain whether and how it influences the ability of the parasite to invade and replicate inside its host cells. Thus, we now propose to elucidate the role of these MTs during infection and to identify essential nutrients used by the parasite during its developmental process. We will accomplish this through two complementary approaches: We will employ RNA interference (RNAi), a technique whose use for the study of malaria liver infection has been pioneered by the host laboratory (2, 3, 22), to systematically knock-down the expression of the DE MT genes encoding aminoacid transporters identified in our microarray study (8) (Fig. 5). This functional genomics analysis will use human hepatoma cells and will be performed in combination with that proposed in Task 2. Genes will be screened in three consecutive RNAi rounds and the resulting infection phenotypes will be assessed through infection measurement methods developed in the host laboratory (18, 19). In parallel, we will perform medium depletion or supplementation studies in which the requirements of the parasite for specific molecules will be addressed. In these studies we will selectively remove or add nutrient molecules to the infected cell growth medium and will evaluate the consequences of the altered availability of those nutrients on infection.
Infection of liver cells by Plasmodium involves the initial traversal of cells by the parasite, followed by invasion of a cell (with formation of a parasitophorous vacuole) and a subsequent extensive replication phase. During the studies described above we will also investigate which of these processes are affected by the treatments employed. To do this, we will use a method developed in the host laboratory that uses flow cytometry to distinguish each of these phases of hepatic infection (18), in parallel with established immunofluorescence methods.
By combining a functional genomics and a nutrient-based approach we expect to identify host MTs and nutrient molecules that influence Plasmodium's infection of hepatocytes and to elucidate how they impact on the parasite's ability to traverse, invade and develop inside its host cells. Host pathways identified in this way will be further dissected through a combination of RNAi- and inhibitor-based studies targeting relevant components of those pathways. When appropriate, in vivo studies with relevant KO rodent models will also be performed and infection measured by quantitative real-time PCR or by a luminescence-based method recently developed in the host lab (19). Overall, these combined strategies will elucidate the mechanism through which the identified host factors influence infection and will inform potential targets for anti-malarial intervention, which will be thoroughly evaluated in Task 6 of the project.
For details on the planned schedule for this task and the allocation of human resources to it the reviewer is kindly referred to the files Timeline.xls and PersonMonths.xls, respectively. The requested budget will cover the acquisition of RNAi reagents, parasite production, cell culture, rodent models acquisition and maintenance, and expenses associated with infection measurement techniques.

**Membros da equipa de investigação nesta tarefa**

Members of the research team in this task

(BTI) Bolseiro Técnico de Investigação (Lic. ou Bacharel) 1; Eliana Patricia Coelho Real; Ghislain Cabal; Miguel Prudêncio; Patrícia dos Santos Meireles;

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| Designação da tarefa<br>Task denomination | Data de início<br>Start date | Data de fim<br>End date | Duração<br>Duration | Pessoas * mês<br>Person * months |
|---|---|---|---|---|
| Ion transport and cell homeostasis in... | 01-01-2012 | 31-03-2014 | 27 | 20,2 |

**Descrição da tarefa e Resultados Esperados**

Task description and Expected results

Besides their role in the uptake of nutrients and structural molecules, membrane transporters (MTs) play essential roles in the ion flow across the cell membrane and in the maintenance of ion homeostasis. Our transcriptomics data have identified several ion transporters that are differentially expressed throughout infection (8). Strikingly, we found that pathways directly associated with regulatory volume increase and decrease are up- and down-regulated during infection, respectively (8). Moreover, we have recently shown that intracellular parasites activate volume-regulated anion channel-like activity in the host cell plasma membrane (9). We now propose to link differential gene expression to altered functional ion transporter activity in Plasmodium-infected liver cells.
This Task will engage Dr. Henry Staines, a consultant and collaborator in this project. Dr. Staines' group has the expertise to carry out the functional studies required to understand fully the role of host transport mechanisms during liver-stage infection, as demonstrated previously (9).
We will use RNA interference (RNAi) to down-modulate the expression of ion transporter genes in hepatoma cells (Fig. 5) and infer how they impact on infection. Functional studies employing specific inhibitors of ion transport molecules will also be performed alongside the RNAi-based studies. Additionally, ion homeostasis will be perturbed by external addition/subtraction of appropriate solutes or by the use of ionchelators and the impact of this perturbation on infection will be assessed. When available, appropriate transgenic rodent models will also be used. In all cases, infection will be monitored through an array of established techniques and novel methodologies developed in the host laboratory (2, 3, 18, 19).
In parallel, Dr. Staines expertise will enable us to carry out electrophysiological studies that will permit the investigation of ion flow across the membrane of individual infected cells. In fact, the scarcity of parasitised liver cells in Plasmodium-infected cell cultures necessitates the use of single-cell assays combined with markers of infection (e.g. fluorescently labelled P. berghei parasites) for physiological studies of this stage. We have already demonstrated successfully the electrophysiological technique of patch-clamp, with single infected cells (9). This technique is the most appropriate choice for the study of ion channels. If appropriate, we can also employ: i) X-ray microanalysis, which allows the measurement of elemental compositions in single whole cells and sections of unstained freeze-dried samples and ii) single-cell fluorescence assays, which in conjunction with laser scanning confocal microscopy allows the measurement of numerous solutes.
We expect that the combination of these approaches will elucidate hitherto unknown aspects of ion homeostasis during plasmodial liver infection. To maximize resources, the functional genetics assays proposed in this Task will be combined with those proposed in

Task 1. Furthermore data obtained here will be analysed in conjunction with data obtained in Tasks 1 and 5 to provide an integrated view of the regulation of homeostasis of infected cells. Finally, we anticipate that results from this task will identify potential druggable targets that will be investigated in Task 6.
For details on the planned schedule for this task and the allocation of human resources to it the reviewer is kindly referred to the files Timeline.xls and PersonMonths.xls, respectively. This Task will also involve resources and expertise from Dr. Staines' group. The requested budget will cover the acquisition of RNAi reagents, parasite production, inhibitors/activators, rodent models, expenses associated with infection measurement techniques and electrophysiological studies. It will also cover travelling expenses between the host and Dr. Staines' laboratories.

**Membros da equipa de investigação nesta tarefa**

Members of the research team in this task

(BTI) Bolseiro Técnico de Investigação (Lic. ou Bacharel) 1; Eliana Patricia Coelho Real; Ghislain Cabal; Miguel Prudêncio; Patrícia dos Santos Meireles;

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| Designação da tarefa<br>Task denomination | Data de início<br>Start date | Data de fim<br>End date | Duração<br>Duration | Pessoas * mês<br>Person * months |
|---|---|---|---|---|
| Glucose uptake by Plasmodium-infected... | 01-06-2012 | 31-05-2014 | 24 | 20 |

**Descrição da tarefa e Resultados Esperados**

Task description and Expected results

Glucose is a crucial source of energy for cells, generating ATP during glycolysis, and an important metabolic intermediate. Glucose can be taken up by cells through several transporter proteins and can be stored in the liver as glycogen (glycogenesis) in an insulin-dependent process. Hepatocytes are able to produce glucose from glycogen following activation of gluconeogenesis.
We have recently shown that plasmodial hexose transporters are essential for the development of Plasmodium liver stages (10). The parasite's requirement for glucose has then been confirmed by preliminary experiments that revealed a correlation between glucose levels in cell growth medium and the extent of Plasmodium's intracellular development (Fig. 2). These observations strongly suggest that glucose plays a crucial role during the liver stage of infection by Plasmodium. However, they also pose new, important questions that we now propose to answer.
One of these questions concerns the source of the glucose used by the Plasmodium liver stages. We will assess infection under conditions of impaired gluconeogenesis to evaluate the impact of glucose production by infected cells on the parasite's fitness. Conversely, we will use fluorescent derivatives of glucose and employ live confocal fluorescence microscopy and FRAP to monitor the uptake of externally added glucose by infected cells. Finally, we will use insulin to promote glycogen accumulation in cells prior to infection and compare their parasite load with that of non-insulin treated cells. We expect that these experiments will elucidate the relative contributions of glucose synthesis and uptake for Plasmodium infection.
Our preliminary results indicate that at least part of the glucose used by the parasite during infection is acquired from extracellular sources. Thus, we will investigate how different host facilitated glucose transporters (GLUTs) in hepatoma cells contribute to the uptake of the glucose used by the parasite. This will be done by using RNAi to independently knock-down the expression of five hepatic GLUTs, GLUT1, GLUT2, GLUT3, GLUT4 and GLUT9 (Fig. 5) and by employing appropriate inhibitors or antibodies to block specific GLUT-mediated uptake. Finally, we will use other hexoses to perform competition assays with glucose and evaluate the resulting impact on infection.
In the experiments outlined above, infection will be monitored through an array of established techniques and novel methodologies developed in the host laboratory (2, 3, 18, 19). Moreover, we will dissect which of the processes that take place during liver infection by Plasmodium (cell traversal, productive invasion, and intracellular development) are affected by the treatments employed. This is particularly relevant since our preliminary data also suggest that glucose depletion may cause a reversible impairment of the parasite's replication (data not shown).
We expect that this Task will elucidate novel aspects of the parasite's modulation of glucose uptake by Plasmodium-infected cells and on the parasite's requirements for glucose throughout infection progress. Given the importance of nuclear receptor-mediated signaling processes in glucose homeostasis, we anticipate that work carried out in this task will be closely articulated with that proposed for Tasks 4 and 5. Finally, if potential targets for anti-malarial intervention are identified in this process, they will be further investigated in Task 6.
For details on the planned schedule for this task and the allocation of human resources to it the reviewer is kindly referred to the files Timeline.xls and PersonMonths.xls, respectively. The requested budget will cover the expenses associated with fluorescence microscopy and flow cytometry, antibody acquisition, cell culture, parasite production, purchase of RNAi reagents and expenses associated with infection measurement techniques.

**Membros da equipa de investigação nesta tarefa**

Members of the research team in this task

(BIC) Bolseiro de Iniciação Científica 1; (BIC) Bolseiro de Iniciação Científica 2; (BTI) Bolseiro Técnico de Investigação (Lic. ou Bacharel) 1; Miguel Prudêncio;

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| Designação da tarefa<br>Task denomination | Data de início<br>Start date | Data de fim<br>End date | Duração<br>Duration | Pessoas * mês<br>Person * months |
|---|---|---|---|---|
| The role of glucose on survival of Pl... | 01-06-2012 | 31-05-2014 | 24 | 21 |

**Descrição da tarefa e Resultados Esperados**

Task description and Expected results

One possibility available to Plasmodium-infected host cells to counter infection is to enter apoptosis, destroying the parasite in the process. Past work has shown that Plasmodium has evolved mechanisms to inhibit host cell apoptosis and thereby ensure its own survival (23, 24). Our preliminary data have shown that the proportion of Plasmodium-infected cells increases with increased availability of glucose (Fig.2), suggesting that glucose specifically enhances survival of infected cells. This can be explained by glucose increasing parasite fitness and its ability to inhibit apoptosis. Thus, we propose to investigate how Plasmodium uses glucose to

develop and promote survival of the host cell.
Initially we will accurately quantify the glucose-dependent increase in survival of hepatoma cells infected with Plasmodium through the use of a GFP-expressing parasite and flow cytometry, in parallel with fluorescence microscopy. This will provide a baseline for the magnitude of the overall influence of glucose on infected cell survival. We will then use TUNEL and activated caspase-3 antibodies to quantify apoptosis of hepatoma cells infected with Plasmodium under different conditions of glucose availability. From a comparison of the data from the two experiments will elucidate the relative contribution of apoptosis inhibition to the overall increase in survival of Plasmodium-infected cells.
Since cells in hepatoma cell lines are immortalized, the experiments described above will be complemented by similar ones conducted on rodent primary hepatocytes. We will then isolate primary hepatocytes not only from wild-type mice but also from caspase 3-deficient mice and compare the effects of glucose on survival of Plasmodium-infected cells. This will provide an independent assessment of the contribution of caspase 3-dependent apoptosis to the overall survival.
Finally, we will carry out experiments where mice fed on controlled glucose diets will be infected by Plasmodium. By analyzing liver slices of these mice by fluorescence microscopy we will be able to infer the relative contributions of glucose to increased cell survival and increased parasite development (Task 3) in an in vivo situation. These experiments may also be carried out using caspase 3-deficient mice to exclude the contribution of caspase 3-mediated apoptosis as well as mouse models for hypo and hyper glycaemia.
The data obtained in Task 4 will intertwine with that generated in Tasks 3 and 5 to understand how Plasmodium subverts its host to fulfill its needs for glucose and how the latter is used to improve the parasite's fitness and increase its chances of survival.
For details on the planned schedule for this task and the allocation of human resources to it the reviewer is kindly referred to the files Timeline.xls and PersonMonths.xls, respectively. The requested budget will cover the acquisition of antibodies and TUNEL reagents, immunofluorescence microscopy and flow cytometry, parasite production, acquisition and maintenance of wild-type and transgenic rodent models, and expenses associated with infection measurement techniques.

**Membros da equipa de investigação nesta tarefa**

Members of the research team in this task

(BIC) Bolseiro de Iniciação Científica 1; (BIC) Bolseiro de Iniciação Científica 2; (BTI) Bolseiro Técnico de Investigação (Lic. ou Bacharel) 1; Miguel Prudêncio;

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| **Designação da tarefa**<br>Task denomination | **Data de início**<br>Start date | **Data de fim**<br>End date | **Duração**<br>Duration | **Pessoas * mês**<br>Person * months |
|---|---|---|---|---|
| Regulation of homeostasis and nutrien... | 01-11-2012 | 31-12-2014 | 26 | 18,6 |

**Descrição da tarefa e Resultados Esperados**

Task description and Expected results

Nuclear receptors (NRs) are ligand-activated transcription factors that control numerous cellular processes, including the transport of ions and molecules across membranes, and glucose metabolism. This makes them key regulatory factors in the liver (15, 25). For these reasons, we postulated that NRs might be active host players during Plasmodium infection of hepatocytes. Thus, we carried out preliminary experiments in which we were able to show that various ligands of NR markedly influence the development of Plasmodium inside hepatoma cells, suggesting that NR and NR-regulated pathways play an essential role during the malaria parasite's liver stage (Fig. 3). Encouraged by these results, we undertook an RNAi screen of the 49 NR in the human genome and showed that down-modulation of several of them led to a significant decrease in infection (Fig. 4). Overall, these preliminary data confirmed that NRs are at play during the liver stage of Plasmodium's life cycle and paved the way for a deeper understanding of how they influence the success of infection. We will start by confirming the results of our preliminary RNAi screen employing alternative methods of infection measurement and quantifying the down-modulation gene expression by quantitative real-time PCR. This will obviate possible false positive or false negative results and provide us with a definitive list of NRs that influence infection. Since one feature of NRs is their ability to bind and sense the presence of small metabolites a set of specific ligands, either endogenous or synthetic, have been identified (and are available) for the majority of them. Thus, we will then proceed to extend our preliminary NR inhibition and activation studies employing suitable antagonists or agonists of selected NRs. Altogether, these experiments will provide a complete picture of the consequences of specific NR activity on Plasmodium liver stage development.Subsequently, we will employ a cell-based reporter assay to determine whether NR transcriptional activity is directly involved in Plasmodium development. In this assay, cultured cells will be transiently transfected with the candidate NR cDNA and a luciferase reporter gene (20). Cells will then be infected with Plasmodium and monitored for NR-dependent luciferase activity. This will also help to distinguish whether NR up- or down-regulate transcription during infection (17). Once activated, some NR are known to be translocated to the nucleus (15). Therefore, the sub-cellular localization of NR upon infection will be assessed by immuno-fluorescence microscopy.Given the pervasive nature of the processes controlled by NR, it can also be expected that the NR characterized in this Task will display transcriptional regulation of the genes and pathways identified in Tasks 1 to 4 of this project. Therefore, the level of expression of those genes will be determined by qRT-PCR upon NR knock-down by RNAi and/or activation/inhibition by agonists/antagonists. Moreover, the cell-based luciferase assay will be used to assess the ability of the nutrients identified in Task 1 to bind and activate the NR previously characterized in the present task.We expect that the results obtained in Task 5 will elucidate the NR-mediated mechanisms that regulate the parasite feeding processes and homeostasis during the liver stage of malaria infection.For details on the planned schedule for this task and the allocation of human resources to it the reviewer is kindly referred to the files Timeline.xls and PersonMonths.xls, respectively. The requested budget will cover the acquisition of antibodies for immuno-fluorescence, NR agonists and antagonists, reagents for luciferase assay and qRT-PCR, parasite production, cell transfection reagents, and expenses associated with infection measurement techniques.

**Membros da equipa de investigação nesta tarefa**

Members of the research team in this task

(BIC) Bolseiro de Iniciação Científica 1; (BIC) Bolseiro de Iniciação Científica 2; Ghislain Cabal; Miguel Prudêncio; Patrícia dos Santos Meireles;

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| **Designação da tarefa** | **Data de início** | **Data de fim** | **Duração** | **Pessoas * mês** |
|---|---|---|---|---|

| Task denomination | Start date | End date | Duration | Person * months |
|---|---|---|---|---|
| Assessment and validation of anti-mal... | 01-01-2013 | 31-12-2014 | 24 | 20 |

**Descrição da tarefa e Resultados Esperados**

Task description and Expected results

Besides revealing novel, fundamental aspects of the biology of hepatic infection by Plasmodium, the present proposal aims to identify potential targets for anti-malarial intervention. In fact, transport proteins are excellent drug targets and make a significant contribution to the relatively small number (< 350) of all known protein drug targets (26). Of the 186 human targets for FDA-approved oral drugs, 24 (13%) are transporters (26). Transporters can also provide delivery routes for drugs that target intracellular processes and are involved in mediating drug resistance. Likewise, glucose transport and metabolism is the target of various drugs, namely for the treatment of diabetes. Finally, the role of nuclear receptors in homeostasis made them major targets for drug discovery for the treatment of various metabolism-related diseases with several of them already being used as clinical drugs (25). Thus, it can reasonably be expected that several druggable targets will be identified during the work carried out in Tasks 1 to 5.
In the present task, we will investigate the potential of the identified host molecules to serve as anti-malarial targets. We will also identify compounds that can be used as inhibitors or modulators of the function of those molecules, prioritizing those which have already been approved for human use in other diseases. We will carry out in vitro studies to test the ability of these compounds to impair infection of hepatoma cell lines by Plasmodium.
The availability of rodent animal models for the study of malaria provides unique opportunities to assess the potential of host molecules to serve as targets for malaria prophylaxis. Once sufficient in vitro data is gathered to warrant the use of animal models, the anti-malarial efficacy of compounds selected in our in vitro studies will be tested in the context of an in vivo infection. Compounds will be administered orally, intra-peritoneally or intra-venously and infection will be monitored as described below. In parallel we will confirm their molecular targets, as predicted in Tasks 1 to 5. Thus, in the cases where this has not already been done in the context of those tasks, we will employ in vivo RNA interference using siRNA formulated in liposomal nanoparticles, a technique that the host laboratory has successfully employed in the past (2-4), and transgenic mice where a specific gene of interest has been deleted.
In all cases, mice will be infected either by intra-venous injection of sporozoites or by mosquito bite. Parasite liver load will be determined either by quantitative real-time RT-PCR or by a luminescence-based method recently published by the host laboratory (19). The appearance of blood-stage parasites will be assessed by daily monitoring of parasitemia (percentage of infected red blood cells) and disease symptoms.
We expect that these approaches will elucidate the suitability of identified host molecules and pathways as malaria prophylactic targets in an in vivo context and will inform new intervention strategies against the disease. Rather than carrying out Task 6 at the end of the project, the experiments proposed here will be undertaken in parallel with the remaining Tasks, as they produce data that warrants the evaluation and validation of potential anti-malarial targets and targeting compounds.
For details on the planned schedule for this task and the allocation of human resources to it the reviewer is kindly referred to the files Timeline.xls and PersonMonths.xls, respectively. Selection of the most appropriate genes to be targeted will engage the expertise of Prof. Maria M. Mota, a consultant in this project, and a world-renowned expert in the field of malaria research. The requested budget will cover the acquisition and maintenance of rodents (including transgenics), liposomal-formulated siRNAs, and inhibitors, as well as expenses associated with parasite production and infection measurement.

**Membros da equipa de investigação nesta tarefa**

Members of the research team in this task

(BIC) Bolseiro de Iniciação Científica 1; (BIC) Bolseiro de Iniciação Científica 2; (BTI) Bolseiro Técnico de Investigação (Lic. ou Bacharel) 1; Ghislain Cabal; Miguel Prudêncio; Patrícia dos Santos Meireles;

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## 3.2.4. Calendarização e Gestão do Projecto

**3.2.4. Project Timeline and Management**

### 3.2.4.a Descrição da Estrutura de Gestão

3.2.4.a Description of the Management Structure

The project will be undertaken through the coordinated efforts of seven researchers (MP, GC, ER, PM, one technician to be hired within the scope of the project and 2 Master's students that will be incorporated at different stages of the project) and two consultants (MMM, HMS). The articulation of the tasks and the relative contributions of each participant are detailed in the attached files Timeline.pdf and PersonMonths.pdf, to which the reviewer is kindly referred.
The project will be coordinated by the PI (MP) and the proposed Tasks will be carried out at the Malaria Unit (UMA) of the Instituto de Medicina Molecular (IMM). The UMA-IMM, led by Prof. Maria Mota, a consultant in this project, has world-renowned expertise in malaria and, in particular, in the study of host factors that influence infection by Plasmodium. The PI is a Staff Scientist at the IMM-UMA, holding a CIÊNCIA 2007 position towards becoming a fully independent researcher, in which is highly supported by the unit leader. He is a liver-stage malaria expert who has written various reviews on this subject and has participated in numerous studies, published in international peer-reviewed journals. He has developed techniques for studying hepatic malaria infection and identifying host molecules that modulate it. Dr. Ghislain Cabal and Dr. Eliana Real are experts in cell biology fluorescence microscopy and microbial infections. Ms. Patrícia Meireles is a PhD student whose work in the project will make up a significant proportion of her thesis. Two Master's students (BIC) and a Technician (BTI) will be hired in the context of this project. Dr. Henry Staines, a consultant in this project and a collaborator of the host laboratory, is an expert in electrophysiological techniques and their application to the study of malaria infection.
Regular meetings will take place between the team members and the consultants to discuss progress and delineate future strategies. It should be emphasised that all the participants in this project have collaborated in the past and that a solid history of cooperation and fruitful discussions exists between them. Thus, informal meetings may also take place between specific members of the team, whenever these may be relevant for the discussion of issues of relevance to the project. Overall, the project has been structured in

such a way that all tasks and the participants involved in each of them have been clearly defined so as to capitalise on each one's expertise and maximise resources and productivity.

### 3.2.4.b Lista de Milestones

3.2.4.b Milestone List

| **Data**<br>Date | **Designação da milestone**<br>Milestone denomination |
|---|---|
| 31-12-2012 | Conclusion of RNAi screens |

**Descrição**
Description

At this point we expect to have carried out all the RNAi analyses proposed in Tasks 1, 2 and 3. This coincides with the initiation of the assessment and validation of anti-malarial targets proposed in Task 6.

| **Data**<br>Date | **Designação da milestone**<br>Milestone denomination |
|---|---|
| 30-09-2013 | Ion transporters and homeostasis |

**Descrição**
Description

This milestone coincides with conclusion of Task 2, at which point we expect to have clarified how the parasite modulates the host's ion transporters to regulate homeostasis of the intracellular environment.

| **Data**<br>Date | **Designação da milestone**<br>Milestone denomination |
|---|---|
| 31-12-2013 | Aminoacid uptake and use |

**Descrição**
Description

This milestone coincides with the expected conclusion of Task 1, at which point we expect to have elucidated the mechanisms through which the Plasmodium parasites engage the host's resources to obtain the aminoacids required for their development.

| **Data**<br>Date | **Designação da milestone**<br>Milestone denomination |
|---|---|
| 31-03-2014 | Glucose uptake and use |

**Descrição**
Description

This milestone coincides with conclusion of Tasks 3 and 4, at which point we expect to understand how glucose is taken up by the host cell and how it influences the parasite's development.

| **Data**<br>Date | **Designação da milestone**<br>Milestone denomination |
|---|---|
| 31-12-2014 | Target identification and validation |

**Descrição**
Description

This milestone coincides with the end of the project, at which point we expect to have identified and validated novel targets for anti-malarial intervention.

### 3.2.4.c Cronograma

3.2.4.c Timeline

*Ficheiro com a designação "timeline.pdf", no 9. Ficheiros Anexos, desta Visão Global (caso exista).*
*File with the name "timeline.pdf" at 9. Attachments (if exists).*

## 3.3. Referências Bibliográficas

**3.3. Bibliographic References**


| **Referência**<br>Reference | **Ano**<br>Year | **Publicação**<br>Publication |
|---|---|---|
| 1 | 2006 | **Prudêncio M, Rodriguez A, Mota MM (2006) "The silent path to thousands of merozoites: the Plasmodium liver stage", Nat. Rev. Microbiol., 4, 849-856** |
| 2 | 2008 | **Prudêncio M, Rodrigues CD, Hannus M, Martin C, Real E, Gonçalves LA, Carret C, Dorkin R, Röhl I, Jahn-Hoffmann K, Luty AJ, Sauerwein R, Echeverri CJ, Mota MM (2008a), "Kinome-wide RNAi screen implicates at least 5 host hepatocyte kinases in Plasmodium sporozoite infection", PLoS Pathog., 4, e1000201** |
| 3 | 2008 | **Rodrigues CD, Hannus M, Prudêncio M, Martin C, Gonçalves LA, Portugal S, Epiphanio S, Akinc A, Hadwiger P, Jahn-Hofmann K, Röhl I, van Gemert GJ, Franetich JF, Luty AJ, Sauerwein R, Mazier D, Koteliansky V, Vornlocher HP, Echeverri CJ, Mota MM. (2008) "Host scavenger receptor SR-BI plays a dual role in the establishment of malaria parasite liver infection", Cell Host Microbe, 11, 271-282** |
| | | **Epiphanio S, Mikolajczak SA, Gonçalves LA, Pamplona A, Portugal S, Albuquerque S,** |

| | | |
|---|---|---|
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| 26 | 2006 | Overington JP, Al-Lazikani B, Hopkins AL. (2006) “How many drug targets are there?”, Nature Rev. Drug Discovery, 5, 993-996 |


### 3.4. Publicações Anteriores
**3.4. Past Publications**


| Referência<br>Reference | Ano<br>Year | Publicação<br>Publication |
|---|---|---|
| [Prudencio2006] | 2006 | Prudêncio M, Rodriguez A, Mota MM (2006) “The silent path to thousands of merozoites: the Plasmodium liver stage”, Nat. Rev. Microbiol., 4, 849-856 |
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## 4. Equipa de investigação
4. Research team

### 4.1 Lista de membros
**4.1. Members list**

| Nome<br>Name | Função<br>Role | Grau académico<br>Academic degree | %tempo<br>%time | CV nuclear<br>Core CV |
|---|---|---|---|---|
| Miguel Prudêncio | Inv. Responsável | DOUTORAMENTO | 40 | ✓ |
| Eliana Patricia Coelho Real | Investigador | DOUTORAMENTO | 20 | ✗ |
| Ghislain Cabal | Investigador | DOUTORAMENTO | 40 | ✓ |
| Patrícia dos Santos Meireles | Investigador | MESTRADO | 75 | ✗ |

*(O curriculum vitae de cada membro da equipa está disponível clicando no nome correspondente)*
*(Curriculum vitae for each research team member is available by clicking on the corresponding name)*

**Total: 4**

### 4.2. Lista de membros a contratar durante a execução do projecto
**4.2. Members list to hire during project"s execution**

| Membro da equipa<br>Team member | Função<br>Role | Duração<br>Duration | %tempo<br>%time |
|---|---|---|---|
| (BIC) Bolseiro de Iniciação Científica 1 | Bolseiro | 12 | 100 |
| (BIC) Bolseiro de Iniciação Científica 2 | Bolseiro | 12 | 100 |
| (BTI) Bolseiro Técnico de Investigação (Lic. ou Bacharel) 1 | Bolseiro | 36 | 100 |

**Total: 3**

## 5. Outros projectos
5. Other projects

### 5.1. Projectos financiados
5.1. Funded projects

| Referência<br>Reference | Título<br>Title | Estado<br>Status |
|---|---|---|
| PTDC/BIA-BCM/71920/2006 | O papel do SR-BI na infecção d... | Em curso |
| PTDC/SAU-MII/099118/2008 | A influência dos ácidos biliar... | Em curso |

*(Os detalhes de cada projectos estão disponíveis clicando na referência correspondente)*

*(Details for each project are available by clicking on the corresponding reference)*

**Total: 2**

### 5.2. Candidaturas similares
5.2. Similar applications

*(Sem Candidaturas Similares)*
*(No Similar applications)*

## 6. Indicadores previstos
6. Expected indicators

-

**Indicadores de realização previstos para o projecto**
**Expected output indicators**

| Descrição<br>Description | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|---|---|---|---|---|---|---|
| **A - Publicações**<br>Publications | | | | | | |
| Livros<br>Books | 0 | 0 | 0 | 0 | 0 | **0** |
| Artigos em revistas internacionais<br>Papers in international journals | 0 | 1 | 2 | 3 | 0 | **6** |
| Artigos em revistas nacionais<br>Papers in national journals | 0 | 0 | 0 | 0 | 0 | **0** |
| **B - Comunicações**<br>Communications | | | | | | |
| Comunicações em encontros científicos internacionais<br>Communications in international meetings | 0 | 1 | 1 | 2 | 0 | **4** |
| Comunicações em encontros científicos nacionais<br>Communications in national meetings | 0 | 0 | 0 | 0 | 0 | **0** |
| **C - Relatórios**<br>Reports | 0 | 0 | 0 | 0 | 0 | **0** |
| **D - Organização de seminários e conferências**<br>Organization of seminars and conferences | 0 | 0 | 0 | 0 | 0 | **0** |
| **E - Formação avançada**<br>Advanced training | | | | | | |
| Teses de Doutoramento<br>PhD theses | 0 | 0 | 0 | 1 | 0 | **1** |
| Teses de Mestrado<br>Master theses | 0 | 1 | 1 | 0 | 0 | **2** |
| Outras<br>Others | 0 | 0 | 0 | 0 | 0 | **0** |
| **F - Modelos**<br>Models | 0 | 0 | 0 | 0 | 0 | **0** |
| **G - Aplicações computacionais**<br>Software | 0 | 0 | 0 | 0 | 0 | **0** |
| **H - Instalações piloto**<br>Pilot plants | 0 | 0 | 0 | 0 | 0 | **0** |
| **I - Protótipos laboratoriais**<br>Prototypes | 0 | 0 | 0 | 0 | 0 | **0** |
| **J - Patentes**<br>Patents | 0 | 0 | 0 | 0 | 0 | **0** |
| **L - Outros**<br>Other | | | | | | |

**Acções de divulgação da actividade científica**
**Scientific activity spreading actions**

The Communication and Training Unit is the Institute's first line of interaction with society. The Unit has dedicated members to address Science and Society activities, in collaboration with national and international partners. A science communication programme has been developed which builds on researchers' initiatives as well as projects aiming at promoting a two-way interaction between researchers and a wide range of audiences. Activities include workshops and hands-on activities for younger generations, meetings involving scientists and different publics, the development of educational resources and media training activities for scientists. Schools and the media are among privileged partners/audiences.

## 7. Orçamento
7. Budget

-

**Instituição Proponente**
Principal Contractor

**Instituto de Medicina Molecular**

| Descrição<br>Description | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|---|---|---|---|---|---|---|
| Recursos Humanos<br>Human resources | 0,00 | 13.300,00 | 16.500,00 | 13.600,00 | 0,00 | **43.400,00** |
| Missões<br>Missions | 0,00 | 1.000,00 | 3.000,00 | 3.000,00 | 0,00 | **7.000,00** |
| Consultores<br>Consultants | 0,00 | 1.000,00 | 1.000,00 | 1.000,00 | 0,00 | **3.000,00** |
| Aquisição de bens e serviços<br>Service procurement and acquisitions | 0,00 | 34.593,00 | 38.500,00 | 38.407,00 | 0,00 | **111.500,00** |
| Registo de patentes<br>Patent registration | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| Adaptação de edifícios e instalações<br>Adaptation of buildings and facilities | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| Gastos gerais<br>Overheads | 0,00 | 3.062,00 | 2.333,00 | 2.271,00 | 0,00 | **7.666,00** |
| **TOTAL DESPESAS CORRENTES**<br>TOTAL CURRENT EXPENSES | **0,00** | **52.955,00** | **61.333,00** | **58.278,00** | **0,00** | **172.566,00** |
| Equipamento<br>Equipment | 0,00 | 25.000,00 | 0,00 | 0,00 | 0,00 | **25.000,00** |
| **Total** | **0,00** | **77.955,00** | **61.333,00** | **58.278,00** | **0,00** | **197.566,00** |

**Instituições Participantes**
Participating Institutions
*(Não se encontram registadas Instituições Participantes para este projecto)*
*(No Participating Institution has been registered for this project)*

**Orçamento Global**
Global budget

| Descrição<br>Description | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|---|---|---|---|---|---|---|
| Recursos Humanos<br>Human resources | 0,00 | 13.300,00 | 16.500,00 | 13.600,00 | 0,00 | **43.400,00** |
| Missões<br>Missions | 0,00 | 1.000,00 | 3.000,00 | 3.000,00 | 0,00 | **7.000,00** |
| Consultores<br>Consultants | 0,00 | 1.000,00 | 1.000,00 | 1.000,00 | 0,00 | **3.000,00** |
| Aquisição de bens e serviços<br>Service procurement and acquisitions | 0,00 | 34.593,00 | 38.500,00 | 38.407,00 | 0,00 | **111.500,00** |
| Registo de patentes<br>Patent registration | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| Adaptação de edifícios e instalações<br>Adaptation of buildings and facilities | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| Gastos gerais<br>Overheads | 0,00 | 3.062,00 | 2.333,00 | 2.271,00 | 0,00 | **7.666,00** |
| **TOTAL DESPESAS CORRENTES**<br>TOTAL CURRENT EXPENSES | **0,00** | **52.955,00** | **61.333,00** | **58.278,00** | **0,00** | **172.566,00** |
| Equipamento<br>Equipment | 0,00 | 25.000,00 | 0,00 | 0,00 | 0,00 | **25.000,00** |
| **Total** | **0,00** | **77.955,00** | **61.333,00** | **58.278,00** | **0,00** | **197.566,00** |

**Plano de financiamento**
Finance plan

**Descrição**

| Description | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|---|---|---|---|---|---|---|
| Financiamento solicitado à FCT<br>Requested funding | 0,00 | 77.955,00 | 61.333,00 | 58.278,00 | 0,00 | **197.566,00** |
| Financiamento próprio<br>Own funding | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| Outro financiamento público<br>Other public-sector funding | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| Outro financiamento privado<br>Other private funding | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | **0,00** |
| **Total do Projecto**<br>Total of the project | **0,00** | **77.955,00** | **61.333,00** | **58.278,00** | **0,00** | **197.566,00** |

## 8. Justificação do orçamento
8. Budget rationale

-

### 8.1. Justificação dos recursos humanos
8.1. Human resources rationale

**Tipo**
Type
(BTI) Bolsa de Técnico de Investigação (Lic. ou Bacharel)

**Nº de pessoas**
No. of persons
1

**Duração (em meses)**
Duration (in months)
36

**Custo envolvido (€) *(calculado)***
Total cost (€) *(estimated)*
26.820,00

**Outros custos (€)**
Other costs (€)
4.236,00

**Justificação do financiamento solicitado**
Rationale for requested funding

In addition to the current members of the research team, one technician will be hired. He/she will be working directly under the PI's supervision, and will be fully dedicated to this project. The technician will be responsible for the production of parasites required throughout the project and will be directly involved in Tasks 1-4 (particularly Tasks 3 and 4) as well as the the in vivo studies to be carried out during Task 6. Other costs pertain Voluntary Social Security and Insurance costs.

**Tipo**
Type
(BIC) Bolsa de Iniciação Científica

**Nº de pessoas**
No. of persons
2

**Duração (em meses)**
Duration (in months)
12

**Custo envolvido (€) *(calculado)***
Total cost (€) *(estimated)*
9.240,00

**Outros custos (€)**
Other costs (€)
3.104,00

**Justificação do financiamento solicitado**
Rationale for requested funding

Besides the designated members of the research team, two young researchers will be involved in the proposed work, which will constitute their Master's thesis projects. They will be directly involved in the glucose-related aspects of the study (Tasks 3 and 4) and will also participate in Tasks 5 and 6. Other costs pertain Voluntary Social Security and Insurance costs.

### 8.2. Justificação de missões
8.2. Missions rationale

**Tipo**
Type
Cursos associados à temática do projecto

**Nº de deslocações**
No. of participations
1

**Local**
Venue
International

**Custo envolvido (€)**
Cost (€)
1.000,00

**Justificação do financiamento solicitado**
Rationale for requested funding

Funding for a relevant training workshop to be attended by the PhD student (PM) is contemplated (1000€). This is intended provide the new member of the team with the theoretical and experimental tools that will be required throughout the duration of the project.

**Tipo**
Type
Participação em congressos

**Nº de deslocações**
No. of participations
6

**Local**
Venue
International

**Custo envolvido (€)**
Cost (€)
6.000,00

**Justificação do financiamento solicitado**
Rationale for requested funding

Funding is requested for the participation of team members in international conferences. Requested funding assumes a total of 6 participations. The assumed cost of each participation is 1000 Euros.

### 8.3. Justificação de consultores
8.3. Consultants rationale

**Nome completo**
Full name

Maria Manuel Dias da Mota

**Instituição**
Institution

Instituto de Medicina Molecular

| Fase do projecto | Custo (€) |
|---|---|
| Project phase | Cost (€) |
| Throughout the project | 0,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

No funding is requested for this consultant

**Página na Internet onde pode ser consultado o CV do consultor**
Web page where the consultant's CV can be accessed

*(Vazio)*
*(Void)*

**Nome completo**
Full name

Henry Staines

**Instituição**
Institution

St George's University of London

| Fase do projecto | Custo (€) |
|---|---|
| Project phase | Cost (€) |
| Throughout the project, particularly during completion of Tasks 2 and 5 | 3.000,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

To access this consultant's CV, please use the URL provided and:
Username: fct2011
Password: FCTGRANT
Funding is requested to cover travelling expenses between the host and the consultant's laboratories. Three such trips are contemplated throughout the project, with an assumed cost of 1000 € per trip.

**Página na Internet onde pode ser consultado o CV do consultor**
Web page where the consultant's CV can be accessed

**http://www.imm.fm.ul.pt/fct2011/1298461452.pdf**

### 8.4. Justificação de aquisição de bens e serviços
8.4. Service procurement and acquisitions

| Tipo | Custo (€) |
|---|---|
| Type | Cost (€) |
| Biological Material | 34.500,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover acquisition, breeding and maintenance of parasites and rodent models. Parasites will be used in all Tasks of the project and rodents will be used mainly, but not exclusively, during Task 6. An estimated 11500€/year, on average, in a total of 34500€ is requested to cover the costs of parasite production, rodent model maintenance and acquisition of transgenic lines of rodent models.

| Tipo | Custo (€) |
|---|---|
| Type | Cost (€) |
| RNA interference | 15.000,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover reagents for RNA interference (RNAi) screening and other RNAi experiments, both in vitro and in vivo. This funding will be allocated for completion of Tasks 1-5, with particular emphasis on Tasks 1, 2 and 3. An estimated total of 15000€ is requested for the acquisition of small interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs), and transfection reagents.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Molecular biology reagents and kits | 21.750,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover the necessary material for analysis of gene expression levels and infection assessments. This funding will be employed in all Tasks of the project. An estimated total of 7250€/year is requested to cover the costs of RNA extraction, cDNA, cloning and qRT-PCR kits, in a total of 21750€.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Antibodies | 4.500,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover expenses with antibody acquisition and/or production. This funding will be employed mainly during completion of Tasks 1 to 5. An estimated 1500€/year, in a total of 4500€ is requested for this purpose.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Flow cytometry | 7.800,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover in vitro infection analyses by flow cytometry. The cost of utilisation of the flow cytometry analysers is 10€/hour. This funding will be employed mainly during completion of Tasks 1 to 4. An estimated 2600€/year, on average, in a total of 7800€ is requested for this purpose.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Confocal microscopy | 10.500,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover confocal microscopy of immunostained samples. The cost of utilisation of the confocal microscope is 11€/hour. This funding will be employed mainly during completion of Tasks 2, 3 and 4.An estimated 3500€/year, on average, in a total 10500€ is requested for this purpose.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Cell culture | 3.000,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover expenses with cell culture reagents and plastic consumables. This funding will be allocated for completion of Tasks 1 to 4. An estimated 1000€/year, on average, in a total 3000€ is requested for the acquisition of consumables such as cell growth media and cell culture plates.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| External services | 1.800,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover expenses with external services such as sequencing blood/serum analyses, etc.. An estimated 600€/year, on average, in a total of 1800€ is requested for this purpose.

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Other reagents | 950,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover expenses with reagents other than those previously listed. This funding is requested for the acquisition of consumables such as agarose, buffers, antibiotics, etc..

| Tipo<br>Type | Custo (€)<br>Cost (€) |
|---|---|
| Common costs | 11.700,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Requested funding will cover expenses with IMM common infrastructural costs. An estimated 3900€/year, on average, in a total of 11700€ is requested for this purpose.

### 8.6. Justificação do Equipamento
8.6. Equipment rationale

### 8.6.1. Equipamento já disponível para a execução do projecto
8.6.1 Available equipment

| Tipo de equipamento<br>Equipment type | Fabricante<br>Manufacturer | Modelo<br>Model | Ano<br>Year |
|---|---|---|---|
| qRT-PCR | Applied Biosystems | 7500 FAST | 2005 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| qRT-PCR | Corbett Research | Rotorgene 6000 | 2006 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Nanodrop | Thermo Scientific | ND-1000 | 2003 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Fluorescence/Bioluminescence Imaging | Caliper LifeSciences | IVIS Lumina | 2007 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Widefield Fluorescence Microscope | Carl Zeiss MicroImaging | Axiovert 200M | 2003 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Confocal microscope | Carl Zeiss MicroImaging | LSM 5 Live | 2007 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Confocal microscope | Carl Zeiss MicroImaging | LSM 710 | 2010 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Confocal microscope | Carl Zeiss MicroImaging | LSM 510 Meta | 2006 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Flow cytometer | BD | LSRFortessa | 2011 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Flow cytometer | BD | FACSCalibur | 2005 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Flow cytometer | BD | FACSAria III | 2011 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Flow cytometer | BD | LSRFortessa | 2011 |
| **Tipo de equipamento**<br>Equipment type | **Fabricante**<br>Manufacturer | **Modelo**<br>Model | **Ano**<br>Year |
| Flow cytometer | BD | FACSAria | 2006 |

### 8.6.2. Discriminação do equipamento a adquirir
8.6.2. New equipment requested

| Tipo de equipamento<br>Equipment type | Fabricante<br>Manufacturer | Modelo<br>Model | Custo (€)<br>Cost (€) |
|---|---|---|---|
| Microplate reader | Tecan | Infinite 200 PRO | 25.000,00 |

**Justificação do financiamento solicitado**
Rationale for requested funding

Funding is requested for a luminescence and fluorescence plate reader. This instrument will be thoroughly employed throughout the project for most in vitro assays performed. It will enable measuring the parasite load of cells infected with luciferase-expressing parasites, and cell proliferation, two crucial parameters of several experiments proposed in Tasks 1 to 5. Furthermore, it will enable measuring blood parasitemias of infected mice employed throughout the project, particularly during Task 6.

### 8.7. Justificação de registo de patentes

8.7. Patent registration

*(Vazio)*
*(Void)*

### 8.8. Justificação de adaptação de edifícios e instalações

8.8. Adaptation of buildings and facilities

*(Vazio)*
*(Void)*

## 9. Ficheiros Anexos

9. Attachments

-

| Nome<br>Name | Tamanho<br>Size |
|---|---|
| **Figures.pdf** | 589Kb |
| **Password.pdf** | 52Kb |
| **PersonMonths.pdf** | 56Kb |
| **Timeline.pdf** | 52Kb |

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**Financiado por fundos estruturais da UE e fundos nacionais do MCTES**