Workshop em Celebração

"60 anos do Prof. Glaucius Oliva"

“30 anos de Cristalografia de Proteínas” IFSC/USP

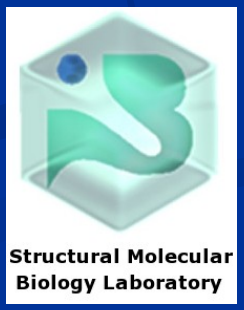
EESC • USP 1977

CIBFar 2019

1988-2019
Crystallography from São Carlos to Botucatu

1986

2019



Arriving in São Carlos - 1986

IFSC USP

1986

18 anos

UFSCar – 1986
Becoming a materialist....

1986/2

Dept. de Ciência de Materiais

Scientific initiation– 1988
USP – Material Sciences

FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

(CRIADA PELA LEI Nº 5.918 DE 18-10-1960)

RUA PIO XI, 1500 - CEP 05060 - TELEFONE: 831-3111 - TELEX: (011) 34615 - ALTO DA LAPA - SÃO PAULO

TERMO DE OUTORGA E ACEITAÇÃO DE BOLSA

OUTORGANTE: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

OUTORGADO: MARCOS ROBERTO DE MATTOS FONTES
CPF: 170450293/49 ENDERECO: DEPTO FISICA CIENCIA MATERIAIS
CX POSTAL 369 - IFQSC/USP
13560 SAO CARLOS-SP

PROCESSO: 87/3136-4

BOLSA DE IC

ORIENTADOR: PROF. DR. MICHEL ANDRE AEGERTER

INSTITUICAO: INST FISICA QUIMICA SAO CARLOS/USP
DEPTO FISICA CIENCIA MATERIAIS

AREA: FISICA DA MATERIA CONDENSADA

PROJETO:
PREPARACAO E CARACTERIZACAO DE SILICA AMORFA ULTRA PURA PELO METODO SOL-GEL.

INICIO DA BOLSA: 01JAN88 TERMINO: 30DEZ88

DURACAO: 12 MESES

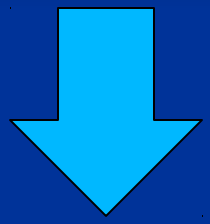
FORMA DE PAGAMENTO: DEPOSITO MENSAL EM CONTA CORRENTE ATE O DIA 15,
A PARTIR DO SEGUNDO MES

VALOR MENSAL: CONFORME TABELA EM VIGOR

RELATORIOS: 30JUN88 E 30DEZ88

DATA DO DESPACHO: 30DEZ87

First interaction with Glaucius
Talking at IFQSC – second semester 1988



Moving the research interest to BIOLOGICAL PHYSICS/BIOPHYSICS

Scientific initiation– 1989
USP – Protein crystallography

Recebido em 15 MAR 1989 FAPESP 15FEV 9 18:14 MX-SIRIUS

FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

(CRIADA PELA LEI Nº 5.918 DE 18-10-1960)

RUA PIO XI, 1500 - CEP 05060 - TELEFONE: 831-3111 - TELEX: (011) 82014 - ALTO DA LAPA - SÃO PAULO

FORMULARIO PARA PARECER INICIAL DA ASSESSORIA PROCESSO 89/0150-1

BOLSA IC
INTERESSADO: MARCOS ROBERTO DE MATTOS FONTES
AREA: FISICA DA MATERIA CONDENSADA
INST FISICA QUIMICA SAO CARLOS/USP
ORIENTADOR PROF. DR. GLAUCIUS OLIVA
PROJETO:
ESTUDOS CRISTALOGRAFICOS DE MIOGLOBINA DE BALEIA DOPADA COM O MARCADOR DE SPIN PARAMAGNETICO ISOTIOCIANATO.

PARECER CUJA COPIA XEROGRAFICA SERA ENVIADA AO INTERESSADO:

O plano de trabalho proposto esta muito bem formulado para iniciar o aluno nas tēcnicas de resolução de estruturas cristalinas, em particular, de proteīnas. O tema proposto, resolução da estrutura de cristais de mioblobina com adição de marcadores de spin, pode dar resultados que viriam a complementar estudos de EPR realizados com esta proteīna.

O aluno apresenta um bom curriculum, com bom desempenho nos cursos, e acredito que tem condições de executar o plano de trabalho. Recomendo a concessão desta bolsa.

Master in Sciences – 1990-1992

UNIVERSIDADE DE SÃO PAULO

INSTITUTO DE FÍSICA E QUÍMICA DE SÃO CARLOS

INTRODUÇÃO AOS MÉTODOS DE DETERMINAÇÃO DE ESTRUTURAS CRISTALINAS POR DIFRAÇÃO DE RAIOS-X: COMPLEXOS DE RUTÊNIO.

MARCOS ROBERTO DE MATTOS FONTES

Dissertação apresentada ao Instituto de Física e Química de São Carlos, USP, para a obtenção do título de ***MESTRE EM FÍSICA APLICADA***

Orientador: Prof. Dr. Glaucius Oliva

Departamento de Física e Ciência dos Materiais

1992

First article

Acta Cryst. (1991). C**47**, 2699–2700

Structure of 1,4-Bis(diphenylphosphinoyl)butane

BY M. R. M. FONTES, G. OLIVA AND J. ZUKERMAN-SCHPECTOR

Instituto de Física e Química de São Carlos, Universidade de São Paulo, Caixa Postal 369, 13560 *São Carlos – SP, Brazil*

AND S. L. QUEIROZ AND A. A. BATISTA

*Departamento de Química, Universidade Federal de São Carlos, Rod. Washington Luiz km*235, 13560 *São Carlos – SP, Brazil*

(*Received* 20 *May* 1991; *accepted* 18 *June* 1991)

Abstract. $C_{28}H_{28}O_2P_2$, $M_r = 458{\cdot}48$, triclinic, $P\bar{1}$, $a = 5{\cdot}826\ (1)$, $b = 8{\cdot}862\ (1)$, $c = 12{\cdot}517\ (2)$ Å, $\alpha = 100{\cdot}29\ (1)$, $\beta = 102{\cdot}67\ (1)$, $\gamma = 104{\cdot}22\ (1)°$, $V = 592{\cdot}5\ (3)$ Å^3, $Z = 1$, $D_x = 1{\cdot}285$ g cm^{-3}, λ(Mo $K\alpha$) = 0·71073 Å, $\mu = 2{\cdot}00$ cm^{-1}, $F(000) = 242$, $T = 296$ K, final $R = 0{\cdot}031$ for 1390 independent observed reflections. The $-(CH_2)_4-$ group is essentially planar with the P atoms 0·126 (1) Å away from its calculated mean plane. Both phenyl rings are planar to within experimental accuracy. The P atom has a distorted tetrahedral configuration.

Experimental. During our studies, using {$RuCl_2$[1,4-bis(diphenylphosphino)]butane} as a starting material for reactions with bulky ligands like triethylphosphite, the title compound was obtained. Single colorless crystals were obtained from CH_2Cl_2/ether by slow evaporation at 293 K. The data collection and refinement parameters are summarized in Table 1. The structure was solved using standard direct methods and difference Fourier techniques. In final cycles of least-squares refinement, all non-H atoms were treated anisotropically, H atoms were refined isotropically. Scattering factors for non-H atoms were taken from Cromer & Mann (1968) with corrections for anomalous dispersion from Cromer & Liberman (1970); for H atoms from Stewart, Davidson & Simpson (1965). Programs used: *SHELX*76 (Sheldrick, 1976) and *ORTEP* (Johnson, 1965).

Table 1. *Crystallographic summary*

Data collection[i]	
Mode	ω–2θ
Scan rate (° min^{-1})	1·8, 5·5
θ range (°)	0–23
Range of hkl	$-6 \le h \le 6$, $-9 \le k \le 9$, $0 \le l \le 13$
Total reflections measured	1848
Unique reflections	1647
R_{int}	0·01
Standard reflections (h^{-1})	1
Variation	None significant
Crystal dimensions approx. (mm)	0·20 × 0·20 × 0·20
Diffractometer	Enraf–Nonius CAD-4, graphite monochromator
Structure determination and refinement[ii, iii]	
Reflections used [$I > 3\sigma(I)$]	1390
No. of variables	202
R, wR	0·031, 0·030
w	$1/[\sigma^2(F_o) + 0{\cdot}0001F_o^2]$
Max. shift/e.s.d.	0·02
Max., min. density in final difference map (e Å^{-3})	0·20, −0·22
S	1·77

Notes: (i) Unit-cell parameters by least-squares refinement of the setting angles of 25 reflections with $12 < \theta < 20°$. (ii) A secondary-extinction correction was applied [$F_{corr} = F_c(1{\cdot}0 \times 10^{-4}\chi F_c^2/\sin\theta)$] where χ refined to 0·009. No correction for absorption. (iii) Function minimized was $\sum w(|F_o| - |F_c|)^2$.

Table 2. *Final atomic coordinates and isotropic temperature factors* (Å^2)

$B_{iso} = (4/3)\sum_i\sum_j \beta_{ij}\mathbf{a}_i.\mathbf{a}_j$

	x	y	z	B_{iso}

Moving back to Protein Crystallography

Glucosamine 6-phosphate deaminase (GlcN6P)

First protein structure solved in South America

PhD – 1992 - 1995

UNIVERSIDADE DE SÃO PAULO
INSTITUTO DE FÍSICA DE SÃO CARLOS
DEP. DE FÍSICA E INFORMÁTICA

Determinação da estrutura cristalográfica da enzima Glucosamina-6-fosfato desaminase de *E. coli* K12 e seus complexos com ativador alostérico e inibidor

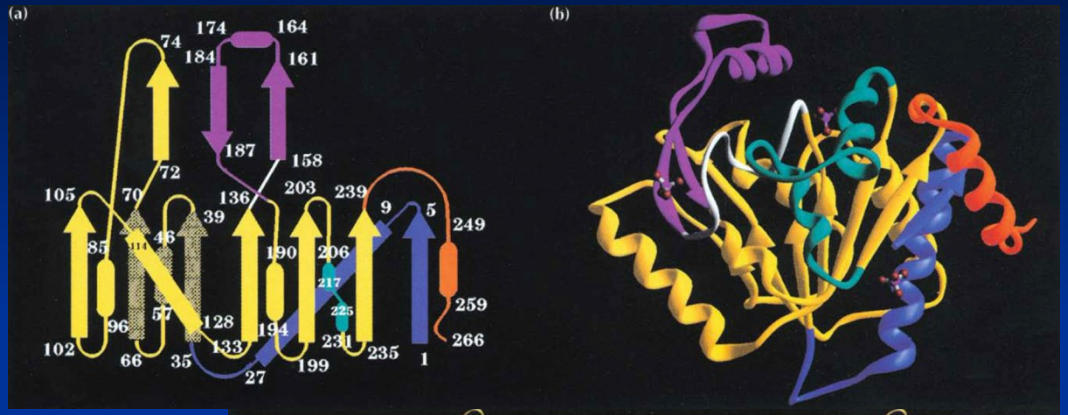
Por: Marcos Roberto de M. Fontes

Orientador: Prof. Dr. Glaucius Oliva

Tese apresentada ao Instituto de Física de São Carlos, para obtenção do título de **Doutor em Física Aplicada**

Agosto de 1995

Glucosamine 6-phosphate deaminase



monomer

hexamer

Glucosamine 6-phosphate deaminase

Structure solved by Multiple Isomorphous Replacement

Need to obtain heavy atom derivatives (several months)

R32 – space group – complicated process

Glucosamine 6-phosphate deaminase

Structure of GlcN6P/allosteric activator

Structure of GlcN6P/inhibitor

Glucosamine 6-phosphate deaminase

Structure and catalytic mechanism of glucosamine 6-phosphate deaminase from *Escherichia coli* at 2.1 Å resolution

Glaucius Oliva[1], Marcos RM Fontes[1†], Richard C Garratt[1], Myriam M Altamirano[2], Mario L Calcagno[2] and Eduardo Horjales[1‡*]

[1]Instituto de Fisica de São Carlos, Universidade de São Paulo, São Carlos, SP, 13560–970, Brazil and [2]Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510, México, D.F., Mexico

Background: Glucosamine 6-phosphate deaminase from *Escherichia coli* is an allosteric hexameric enzyme which catalyzes the reversible conversion of D-glucosamine 6-phosphate into D-fructose 6-phosphate and ammonium ion and is activated by *N*-acetyl-D-glucosamine 6-phosphate. Mechanistically, it belongs to the group of aldose-ketose isomerases, but its reaction also accomplishes a simultaneous amination/deamination. The determination of the structure of this protein provides fundamental knowledge for understanding its mode of action and the nature of allosteric conformational changes that regulate its function.

Results: The crystal structure of glucosamine 6-phosphate deaminase with bound phosphate ions is presented at 2.1 Å resolution together with the refined structures of the enzyme in complexes with its allosteric activator and with a competitive inhibitor. The protein fold can be described as a modified NAD-binding domain.

Conclusions: From the similarities between the three presented structures, it is concluded that these represent the enzymatically active R state conformer. A mechanism for the deaminase reaction is proposed. It comprises steps to open the pyranose ring of the substrate and a sequence of general base-catalyzed reactions to bring about isomerization and deamination, with Asp72 playing a key role as a proton exchanger.

Structure 15 December 1995, **3**:1323–1332

Key words: aldose-ketose isomerase, α/β open structure, allosteric enzyme, NAD-binding domain

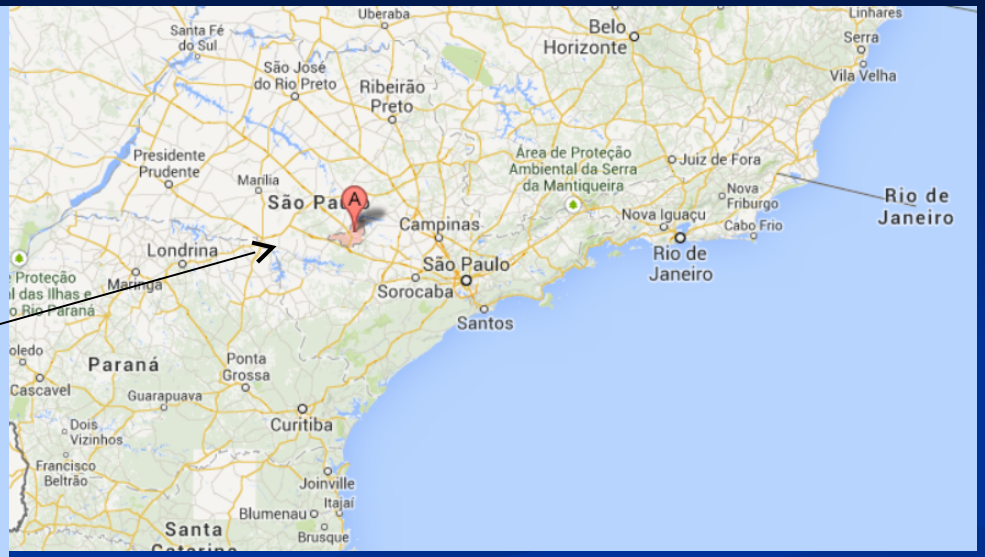
Introduction

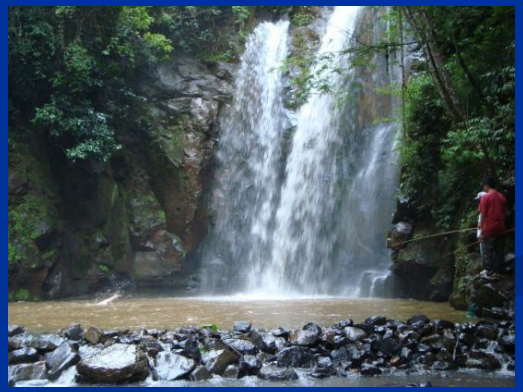
The enzyme glucosamine 6-phosphate deaminase (GlcN6P deaminase, E.C. 5.3.1.10) catalyzes the reversible isomerization and deamination of D-glucosamine 6-phosphate (GlcN6P) into D-fructose 6-phosphate (Fru6P) and ammonium ion [1–3]. This enzyme has been identified in several animal, fungal and bacterial species and completely purified to homogeneity from *Escherichia coli* [3], *Candida albicans* [4] and dog kidney [5]. The gene encoding the enzyme has been cloned from both *E. coli* [6] and *C. albicans* [4]. In *E. coli*, GlcN6P deaminase is an allosteric enzyme, activated by *N*-acetyl-D-glucosamine 6-phosphate (GlcNAc6P). It catalyzes a step in the catabolism of amino sugars, that allows bacteria to utilize glucosamine (GlcN) or *N*-acetyl-D-glucosamine (GlcNAc) from the medium as sources of carbon. Amino sugars are also components of lipopolysaccharide and proteoglycan that form the bacterial cell wall. When no

mediated by GlcNAc6P which binds to the product of the *nagC* gene — a repressor protein. GlcNAc6P, the co-inducer of the regulon, is also the allosteric activator of GlcN6P deaminase, the product of the *nagB* gene. This enzyme, therefore, plays a central role in the regulated balance of amino sugar synthesis and utilization due to its allosteric properties.

GlcN6P deaminase is a hexameric protein composed of identical subunits of 266 residues whose sequence is known from its encoding gene. A systematic search for sequence homology did not reveal significant similarity with any other protein, except with GlcN6P deaminases from other species [10]. The enzyme from *E. coli* has been purified from an overproducing strain [11] and the kinetics of its allosteric activation have been studied in detail [12]; it displays homotropic cooperativity towards its substrates GlcN6P and Fru6P in the forward and

Botucatu-SP-Brazil





Structural Molecular Biology Lab.

1994-2019 (25 years)

Just a desktable and a chair...

No Laboratory

No internet

No infrastructure and chemicals

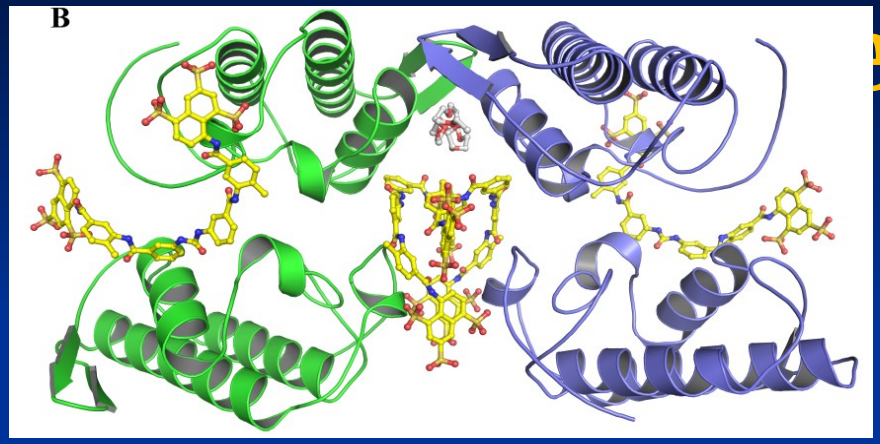
Full Professor Exam - 2010

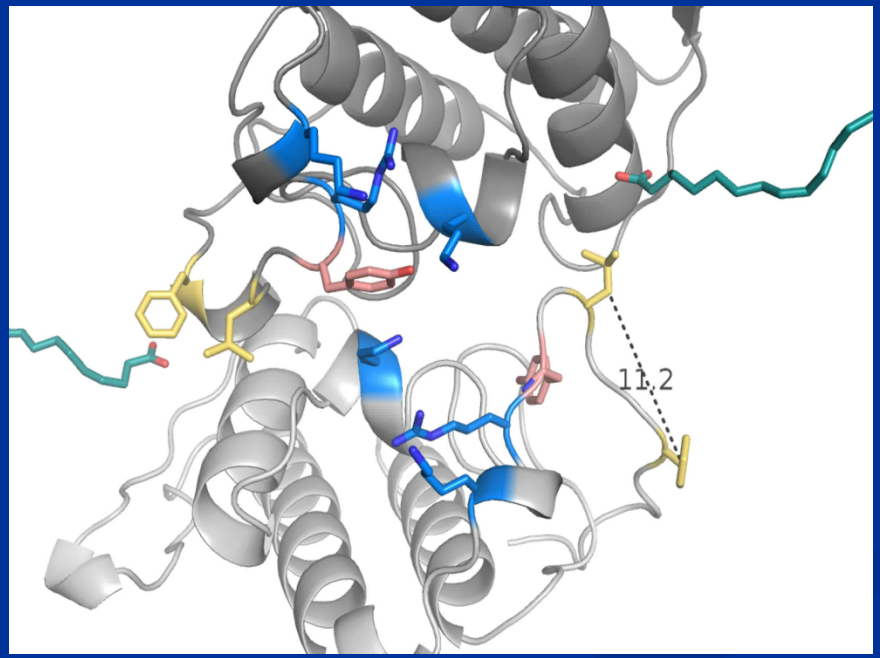
Full Professor Exam - 2010

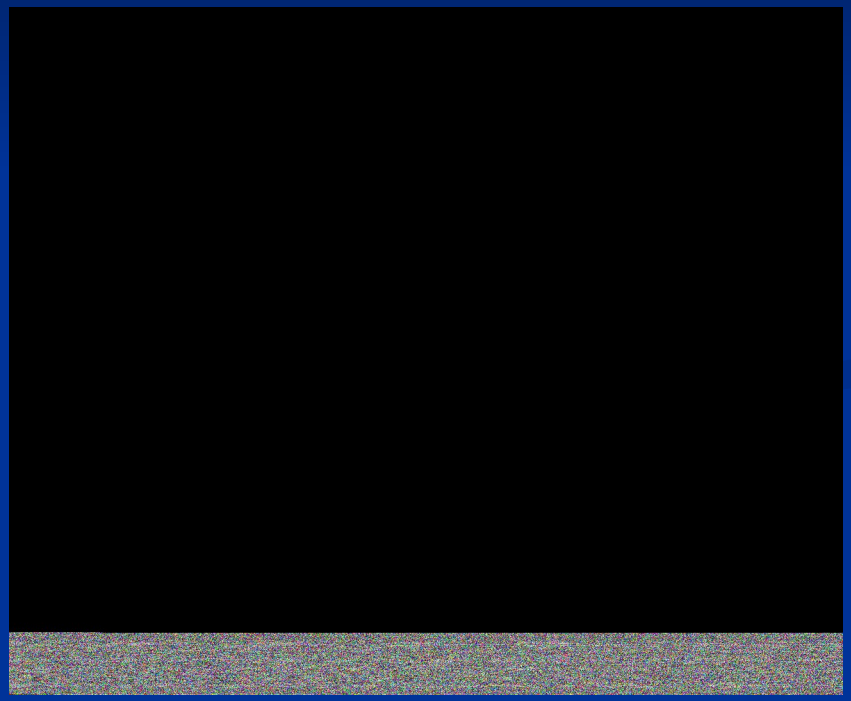
Many research fields…

- Snake venom toxins (PLA_2, PLA_2s inhibitors metaloproteases, serino-proteases, L-aminoacid oxidades, lectins).
- Nuclear import proteins from different organisms
- Fungi glycogen metabolism related proteins
- Trypanosomatid parasite proteins, Telomeric proteins

Several crystal structures ed…







Papers published, citations, grants...

- ~125 articles published
- > 3100 citations
- Grants: 11 FAPESP, 5 CNPq, 4 FINEP, 2 CAPES, 1 INCT
- Well equipped laboratory

But the most important:
people ~100

Carlos A. H. Fernandes

Andréa Coelho de Barros

Guilherme H. M. Salvador

Rafael Junqueira Borges

Fábio Florença Cardoso

Antoniel A S Gomes

Hamine Crisitna de Oliveira

Carlos Natal Jr.

Bruna Zamboni

Cintia Fukuda

Tainá D Silva

Aleff F Francisco

Ivan R Moraes

Cintia Alves

Ana Júlia Levada

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Esther C dos Reis

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Luiz Augusto Bovolenta

Alisson Buchi

Mabel C O da Silva

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Patrícia S Shimabuku

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Luiz E Monteiro

Heitor Katsuyana

André C Fernandes

Andrea D Jacob

Professors / Researches

Prof Angelo Magro

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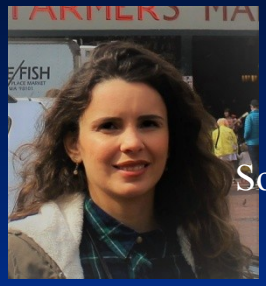
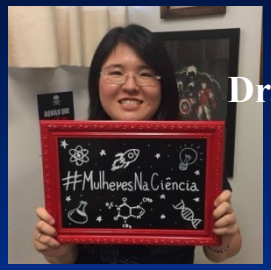


Dr. Natália Bernardes

University of Texas Southwestern Medical Center

Dallas - USA



Dr. Agnes Takeda

Post-doc

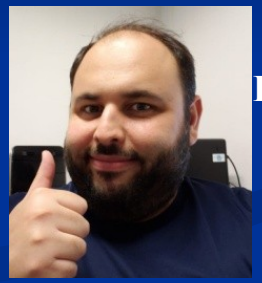
Profa Daniela Marchi

UFPB

Dr. Rafael Borges

University of Barcelona

Spain



Dr. Fábio Cardoso

Post-doc

Prof Walter Cavalcante

UFMG

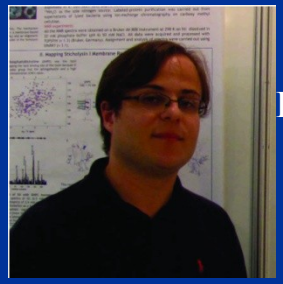
Dr. Juliana dos Santos

Forensics expert

Post-doc

Dr. Guilherme Salvador

Post-doc



Dr. Carlos Fernandes

ENSC - França

Dr. Thiago Dreyer

Forensics expert

Post-doc

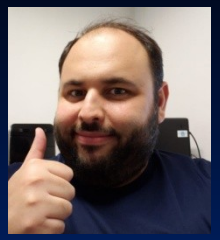
Dr. Andrea de Barros

Post-doc

Current members:

Structural Molecular Biology Laboratory

Marcos Fontes



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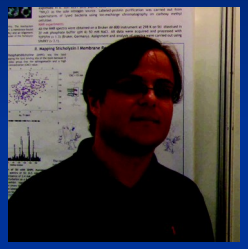

Carlos Fernandes

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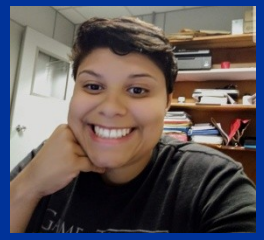
Micaela

Carlos Natal

Cíntia Fukuda



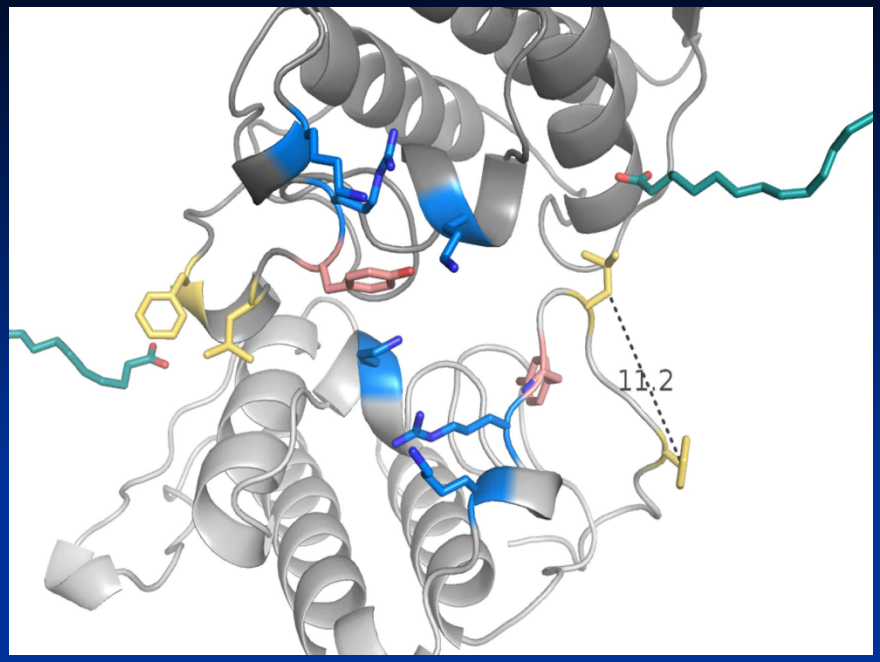
Tainá Dorte

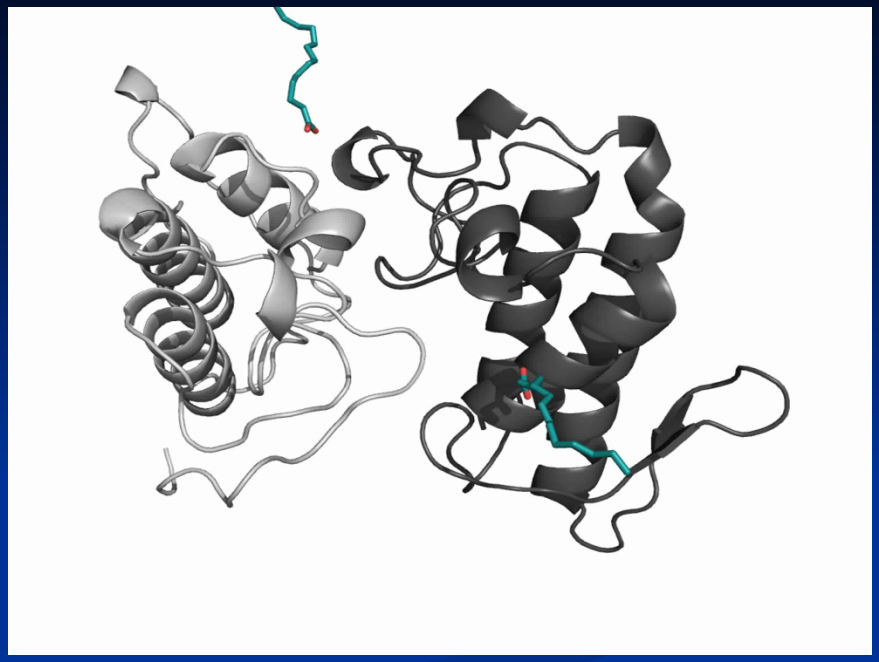
Ana Júlia Levada

Ivan Moraes

Cíntia Alves

Current members





Obrigado e parabéns
GLAUCIUS