TO MAGNIFICO RETTORE OF UNIVERSITA' DEGLI STUDI DI MILANO ID CODE 4456

I the undersigned asks to participate in the public selection, for qualifications and examinations, for the awarding of a type B fellowship at **Dipartimento di Bioscienze**

Scientist- in – charge: Prof. Carlo Camilloni

Mariana Raquel Bunoro Batista

CURRICULUM VITAE

PERSONAL INFORMATION

Surname	Bunoro Batista
Name	Mariana Raquel
Date of birth	30, January, 1989

PRESENT OCCUPATION

Appointment	Structure
Postdoctoral Researcher	University of São Paulo

EDUCATION AND TRAINING

Degree	Course of studies	University	year of achievement of the degree
Degree	Biomolecular and Pysical Science	University of São Paulo	2010
Master	Applied Physics	University of São Paulo	2013
PhD	Chemistry	State University of Campinas	2016

REGISTRATION IN PROFESSIONAL ASSOCIATIONS

Date of registration	Association	City
2017	Brazilian Biophysical Society	Brazil

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FOREIGN LANGUAGES

Languages	level of knowledge
Portuguese	Native
English	Fluent

AWARDS, ACKNOWLEDGEMENTS, SCHOLARSHIPS

Year	Description of award
2017-present	FAPESP Post-doctoral Scholarship
2013-2016	FAPESP Doctoral Scholarship
2011-2013	FAPESP Master Scholarship

TRAINING OR RESEARCH ACTIVITY

Description of Activity

Postdoctoral Research (2017 – present): During my postdoctoral, I have been involved in different projects, as listed above:

1. *"Molecular dynamics simulations of proton dependent oligopeptide transporters"* (main project) – In this project, we intend to investigate the mechanistic details of peptide transport across membranes via a combined experimental-theoretical approach. This project is in collaboration with the group of Prof. Anthony Watts, in charge of the solid state NMR experiments, and the group of Prof. Simon Newstead, responsible for functional assays and crystallizations studies, both from the Biochemistry Department of Oxford University. Proteins involved in peptide uptake and transport belong to the proton-coupled oligopeptide transporter (POT) family. POTs operate through an alternate access mechanism, where the membrane transporter undergoes global conformational changes, alternating between inward-facing (IF), outward-facing (OF), and occluded (OC) states. Conformational transitions are promoted by proton and ligand binding; however, due to the absence of crystallographic models of the OF state, the role of H^+ and ligands is still not fully understood. My research focuses on the characterization of the structure and dynamics of POTs and the investigation of conformational changes of the transporters in response to proton gradients using enhanced sampling MD simulations and constant-pH simulations. In a recently publised work, making use of ABF molecular dynamics simulations and simulating disitinct protonations states of PepTst (a member of POT family) we obtained structural models for the OF conformation and described the accessible conformations in the presence/absence of proton and ligand (JCTC, v. 15, p. 6433-6443, 2019). Currently, my work is focused on the aplication of constant-pH MD simulations of Apo and Holo-PepTst to extend the proton coupled conformational changes studies and provide a molecular description to explain the distinct transport mechanisms associated with di- and tripeptide translocation, observed experimentally.

2. *"Conformational flexibility of GRASP protein and its constituent PDZ subdomains"* - Golgi Reassembly and Stacking Proteins (GRASPs) play pivotal roles in the maintenance of Golgi structure as well as in unconventional protein secretion. These proteins are composed by two structurally similar PDZ domains. Despite their structural similarity, functional asymmetry of the PDZs and constrasting promiscuity of interactions with binding partners are observed *in*

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vivo. In this project, developed in collaboration with other members of the laboratory and Prof. Anthony Watts and Prof. Christina Redfield, from Oxford University, we combined high-field solution-state nuclear magnetic resonance (NMR), synchrotron radiation circular dichroism (SRCD), conventional circular dichroism (CD), steady-state fluorescence and molecular dynamics (MD) simulations to examine the structure, flexibility and stability of the two constituent PDZ domains and suggest an explanation for the higher PDZ1 promiscuity. My particular work consisted in using MD simulations and Elastic Network Models to explain the diffential behavior bewteen PDZs observed experimentaly. Simulations of each PDZ in water and in different concentration of urea and ENM analysis reinforce the hypothesis that PDZ1 is more unstable and flexible than PDZ2 suggested by NMR and CD experiments. Performing free energy calculations of Apo- and Holo-GRASP, we also demonstrated that, after binding, the protein partner significantly reduces the conformational space that GRASPs can access, by stabilizing one particular conformation, in a partner depedent fashion (FEBS. J. Under Review). Moreover, as a postdoc I'm also responsible for training one master student. In his project, we are aplying conventional and umbrella sampling MD simulations to describe the mechanistic details of the process of membrane anchoring of GRASPs via the myristoyl group, attached at the N-terminal of the protein.

3. "T*he effects of nicotine on the thermodynamics and structure of DPPC membranes*" - Biological membranes consist of complex mixtures of proteins, carbohydrates and a wide variety of lipids with different hydrocarbon chains, polar groups, backbone structures, type of chemical linkages, etc. Because of their complexity, studying biological membranes remains a major challenge from both experimental and theoretical perspectives. In this project, we seek to study how small molecules such as drugs alter the thermodynamics and the structure of model membranes. In particular, the drug of choice is nicotine, a natural product found in the nightshade family of plants, which is a potent parasympathomimetic stimulant acting as an agonist at most nicotinic acetylcholine receptors. To this end, we are combining experimental techniques (differential scanning calorimetry and electron paramagnetic resonance) and molecular dynamics simulations. My work consists in the analysis of how nicotine affects the structure of the lipid bilayer using atomistic MD simulations. To evaluate the interaction between nicotine and the membranes we are performing MD simulations of different nicotine/lipid molecular ratios, different membrane compositions and nicotine protonation states and calculating structural parameters, such as number of contatcs, area per lipid, order parameter for lipid hydrophobic chains and mass density profiles.

PhD Research project (2013 - 2016):

1. "Computer simulations of helix 12 conformational free energy profiles of Peroxisome Proliferator Actived Receptors" - Nuclear hormone receptors (NR) are transcription factors that activate gene expression in response to ligands and cofactors. Structural and functional studies of the ligand binding domains (LBD) of NRs revealed that the dynamics of their C-terminal helix (H12) is fundamental for NR activity. In this project, which were an extension of the studies developed during my master, we described the H12 conformational equilibrium through an energetic perspective. Using enhanced sampling MD simulations and free energy calculations (ABF) we studied the ligand and cofactors related conformational changes on Nuclear Receptors. Our results demonstrated that ligand binding stabilizes the agonist H12 conformation relative to other structures, promoting a conformational selection. Similar effects are observed with coactivator association. On the other hand, corepressor binding induces a conformational transition in the protein. These results provide a comprehensive picture of the H12 motions and their functional implications, with molecular resolution (J. Phys. Chem. B, v. 119, p. 15418-15429, 2015).

Master Research project (2011 – 2013):

1. *"Nuclear receptor's helix 12 mobility: comparison between molecular dynamics simulations and fluorescence anisotropy experiments"* - Nuclear hormone receptors (NRs) are major targets for pharmaceutical development. Many experiments demonstrate that their C-terminal Helix (H12) is more flexible in the ligand-binding domains (LBDs) without ligand, this increased mobility being correlated with transcription repression and human diseases. In this work we aimed to describe the H12 motions in solution using molecular dynamics simulations. To bridge the gap between experiments and molecular models we developed a methodology to provide a direct comparison between time resolved fluorescence anisotropy experiments and modeled anisotropy decays. Using this methodology and extensive molecular dynamics simulations of PPAR-gamma LBD, in which the H12 was bound to a fluorescent probe, we showed that the experimental decays rates are dependent on the interactions of the probe with the surface of the protein. Nevertheless, for the probe to interact with the surface of the LBD, the H12 must be folded over the body of the LBD. Therefore, the molecular mobility of the H12 should preserve the globularity of the LBD. These results advanced the comprehension of both ligand-bound and ligand-free receptor structures in solution, and also guided the interpretation of time-resolved anisotropy decays from a molecular perspective, particularly by the use of simulations (Biophysical Journal, v. 105, p. 1670-1680, 2013).

PROJECT ACTIVITY

Year	Project
2017-present	Molecular dynamics simulations of proton dependent oligopeptide transporters
2017-present	Conformational flexibility of GRASP protein and its constituent PDZ subdomains
2019-present	The effects of nicotine on the thermodynamics and structure of DPPC membranes
2013-2016	Computer simulations of helix 12 conformational free energy profiles of Peroxisome Proliferator Actived Receptors
2011-2016	Nucler receptor's helix 12 mobility: comparison between molecular dynamics simulations and fluorescence anisotropy experiments

CONGRESSES AND SEMINARS

Date	Title	Place
2019	**Oral Presentation:** "Mechanism and Energetics of the Transport Cycle in Proton Coupled Oligopeptide Transport"	XLIV Annual Meeting of the Brazilian Biophysical Society.. Santos, Brazil
2019	**Oral Presentation:** "Exploring conformational transitions and free energy profile of proton coupled oligopeptide transporters".	12th EBSA, 10th ICBP-IUPAP Biophysics congress. Madrid, Spain
2018	**Poster Presentation: "**Exploring conformational transitions and free	XLIII Annual Meeting of the Brazilian Biophysics Society. Santos, Brazil

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	energy profile of proton coupled oligopeptide transporters”	
2017	**Poster Presentation:**” Molecular dynamics simulations of proton dependent oligopeptide transporters”	XLII Annual Meeting of the Brazilian Biophysics Society. Santos, Brazil.
2017	**Poster Presentation:** “Molecular dynamics simulations of proton dependent oligopeptide transporters.”	19th IUPAB and 11th EBSA Congress. Edimburgh, Scotland
2016	**Poster presentation:** **”**Conformational diversity of helix 12 of the LBD of PPARγ and functional implications.”	III CCES workshop and SAIMS. Campinas, Brazil
2016	**Mini course presentation:** “Free Energy perturbation.”	II CCES workshop and SAIMS. Campinas, Brazil
2015	**Oral presentation:** “Conformational free energy profile of PPARγ helix 12.”	Brazilian school of molecular modeling. Santo André, Brazil
2014	**Poster presentation:** “Molecular movements and conformational free energy profile of PPARγ helix 12.”	10th Triennial Congress of the World Association of Theoretical and Computacional Chemists – WATOC. Santiago, Chile
2013	**Poster presentation:** “Dynamics of nuclear receptors helix 12 switch of transcription activation by modeling time resolved fluorescence anisotropydecays.”	pDynamo Workshop. São Paulo, Brazil
2012	**Poster presentation:** “Mobility of nuclear receptors helix 12: comparison between molecular dynamics simulations and fluorescence anisotropy experiments.”	NSF Workshop on multiscale modeling and simulation. Montevideo, Uruguai
2011	**Poster presentation:** “Computational study of the temperature dependence of solvation of amino acid side chains using FEP: implications in the protein thermostability”	Brazilian symposium of theoretical chemistry. Ouro Preto, Brazil

PUBLICATIONS

Articles in reviews
MENDES, L. F. S.; **BATISTA, M. R. B.;** JUDGE, P. J.; WATTS, A.; REDFIELD, C.; COSTA-FILHO, A. J. Conformational flexibility of GRASP protein and its constituent PDZ subdomains reveals structural basis of its promiscuous interactome. FEBS Journal. Under Review. 2019.
BATISTA, M. R. B.; WATTS, A.; COSTA-FILHO, A. J. Exploring conformational transitions and free energy profile of proton coupled oligopeptide transporters. Journal of Chemical Theory and Computation, v. 15, p. 6433-6443, 2019.
BATISTA, M. R. B.; MARTÍNEZ, L. Conformational Diversity of the Helix 12 of the Ligand Binding Domain of PPAR and Functional Implications. Journal of Physical Chemistry. B, v. 119, p. 15418-15429, 2015.

BATISTA, M. R. B.; MARTÍNEZ, L. Dynamics of Nuclear Receptor Helix 12 Switch of Transcription Activation by Modeling Time-Resolved Fluorescence Anisotropy Decays. Biophysical Journal, v. 105, p. 1670-1680, 2013.

Congress proceedings

BATISTA, M. R. B.; WATTS, A.; COSTA-FILHO, A. J. Mechanism and Energetics of the Transport Cycle in Proton Coupled Oligopeptide Transport. XLIV Annual Meeting of the Brazilian Biophysics Society. Santos, Brazil, 2019.

BATISTA, M. R. B.; WATTS, A.; COSTA-FILHO, A. J. Exploring conformational transitions and free energy profile of proton coupled oligopeptide transporters. 12th EBSA, 10th ICBP-IUPAP Biophysics congress. Madrid, Spain, 2019.

BATISTA, M. R. B.; WATTS, A.; COSTA-FILHO, A. J. Exploring conformational transitions and free energy profile of proton coupled oligopeptide transporters. XLIII Annual Meeting of the Brazilian Biophysics Society. Santos, Brazil, 2018.

BATISTA, M. R. B.; WATTS, A.; COSTA-FILHO, A. J. Molecular dynamics simulations of proton dependent oligopeptide transporters. XLII Annual Meeting of the Brazilian Biophysics Society. Santos, Brazil, 2017.

BATISTA, M. R. B.; WATTS, A.; COSTA-FILHO, A. J. Molecular dynamics simulations of proton dependent oligopeptide transporters. 19th IUPAB and 11th EBSA Congress. Edimburgh, Scotland, 2017.

BATISTA, M. R. B.; MARTINEZ, L. Molecular movements and conformational free energy profile of PPARγ helix 12. 10th Triennial Congress of the World Association of Theoretical and Computacional Chemists – WATOC. Santiago, Chile, 2014

BATISTA, M. R. B.; MARTINEZ, L. Computational study of the temperature dependence of amino acids side chain solvation: implications fot protein thermal stability. XVI Brazilian Symposium of Theoretical Chemistry. Ouro Preto, Brazil, 2011.

OTHER INFORMATION

RELEVANT EXTRACURRICULAR COURSES

1. **2018** - Hands-on Workshop on Enhanced Sampling and Free-Energy Calculation. University of Illinois, Urbana, USA.

2. **2015** - Practical Docking. UFABC, Santo André, Brazil.

3. **2015** - Hybrid QM/MM methods to study chemical and enzymatic reactions. UFABC, Santo André, Brasil.

4. **2015** - Systematic and accurate coarse-grained of soft matter and biological system, UFABC, Santo André, Brazil.

5. **2013** - pDynamo workshop, University of São Paulo, São Paulo, Brazil.

6. **2012** - NSF Workshop on multiscale modeling and simulation. Instituto Pasteur de Montevideo, Montevideo, Uruguai.

7. **2012** - Monte Carlo Simulations. Physics Institute of São Carlos, São Carlos, Brazil.

Declarations given in the present curriculum must be considered released according to art. 46 and 47 of DPR n. 445/2000.

The present curriculum does not contain confidential and legal information according to art.

4, paragraph 1, points d) and e) of D.Lgs. 30.06.2003 n. 196.

Place and date: Ribeirão Preto, 20/12/2019

SIGNATURE

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