Gabriel Monteiro da Silva

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Education

- Ph.D., **Molecular Biology and Biochemistry** – Brown University, Rhode Island/USA (2019 – **Ongoing,** expected Summer 2024)

Thesis advisor: **Prof. Brenda Rubenstein**

- M.Sc., **Genetics**, FMRP – Universidade de São Paulo, Ribeirão Preto/Brazil (2016 – 2018)

Dissertation Advisor: **Prof. Silvana Giuliatti**

- B.Sc., **Pharmacy and Biochemistry**, FCFRP – Universidade de São Paulo, Ribeirão Preto/Brazil (2011 – 2016)

Research Experience

Blavatnik Family Fellow / Ph. D. Candidate – MCB, **Brown University** Mar 2020 - Ongoing

➢ **Method development for the high-throughput prediction of the conformational landscapes of proteins and their variants**

Collaborators: Prof. George Lisi (Brown U.)**,** Prof. Lorin Crawford (Microsoft), Prof. Dan DiMaio (Yale U.)

- Developed statistical learning-empowered methods for the efficient exploration of the conformational landscapes of proteins
- Modified the protein structure prediction algorithm AlphaFold2 (AF2) to accurately predict the major conformational states of proteins, as well as their respective populations
- Our AlphaFold2 version achieves ensemble prediction by systematic subsampling of the co-evolutional signal used as input, and can efficiently predict changes in the conformational landscape of proteins in response to mutations
- This application is entirely novel and represents a significant reduction in computational cost compared to previous methods, with key implications for drug design, protein engineering, and basic biomedical discovery
  - This work culminated in a manuscript recently submitted to *Nature Communications*, also available as a pre-print at bioRxiv (doi: 10.1101/2023.07.25.550545)

Related Publications:

- Predicting Relative Populations of Protein Conformations without a Physics Engine Using AlphaFold2. bioRxiv (2023)
- A topological data analytic approach for discovering biophysical signatures in protein dynamics." *PloS Comp. Bio* (2022)

➢ **Investigating the effects of resistance-granting mutations in the conformational landscapes and active sites of enzymes**

Collaborators: Dr. David Dalgarno (Dalgarno Scientific, LLC), Prof. Daniel Weinreich (Brown U.)**,** Prof. Marty Ytreberg (U. of Idaho)

- Explored the relationship between inhibitor resistance and changes in conformational state equilibriums in mutant enzymes
- Used enhanced sampling molecular dynamics simulations methods to rigorously sample pathways and to systematically measure their relative populations compared to controls with known potential energy landscapes
- Discovered a collection of six Abl1 kinase mutations that grant inhibitor resistance by stabilizing the active form of the enzyme, which poorly binds most kinase inhibitors
- The shared mechanism behind all six mutations facilitates **polypharmacology** and the development of pan-variant inhibitors
- These results bridge a longstanding gap in knowledge in the field: three of the six mutations occur in residues that are relatively distant to the Abl1 active site, and their effects on binding were puzzling before our observations
  - A manuscript detailing these results and their implications for drug discovery is currently in preparation.
- I also measured the effects of resistance-granting mutations in the microenvironment of the active sites of mutant enzymes
- Using covalent docking and binding pose metadynamics, I systematically measured the effects of 20 substitutions in the binding affinities of over 90 different beta-lactam antibiotics against **TEM beta-lactamase**,
- I identified two mutations that alter TEM's specificity, allowing it to strongly bind latest generation cephalosporins
- These mutations involve the substitution of a conserved alanine by a comparatively bulkier arginine or lysine, sharing a common mechanism for provoking drug resistance by changing the active site microenvironment
- This shared mechanism also presents an opportunity for orienting the rational design of next generation antibiotics

Related Publications:

- Covalent docking and molecular dynamics simulations reveal the specificity-shifting mutations Ala237Arg and Ala237Lys in TEM beta-lactamase *PloS Comp. Bio* (2022)

Key technologies: Molecular modeling (Schrödinger Prime, AlphaFold2), enhanced sampling molecular dynamics simulations (GROMACS, Desmond, OpenMM, Weighted Ensemble [WESTPA], Metadynamics [PLUMED], Gaussian-accelerated methods [GAMD]), Markov State Models (PyEMMA, Enspara), Molecular docking (Schrödinger Glide Induced Fit Docking, CovDock, ensemble docking with AutoDock Vina/Smina, free energy perturbations (GROMACS with PyAutoFEP) umbrella sampling (GROMACS/OpenMM), neural networks (DeepChem, ChemProp, or TensorFlow directly)

Postgraduate Associate – Pediatrics, **Yale School of Medicine** June 2018 – July 2019

Principal Investigator: **Prof. Patrick Gallagher**

- **Modeling backbone effects of rare mutations in the human glucose-6-phosphate isomerase enzyme**

Key technologies: Molecular modeling (PyMol, Modeler), MD simulations (GROMACS, Amber 18), cloning, site-directed mutagenesis, tissue culture, minimum inhibitory concentration assays, mRNA rescue experiments

M.S. Candidate – Ribeirão Preto Medical School (FMRP), Universidade de Sâo Paulo, Brazil August 2016 – June 2018

Principal investigator: **Prof. Silvana Giuliatti**

- **Virtual Screening against Oncoprotein E6 from high-risk Human Papillomavirus variants**
- **Prediction of the therapeutic potential of a variety of natural products derived from South American flora**

Key technologies: Molecular modeling (PyMol), molecular docking (Gold, AutoDock), MD simulations (GROMACS)

Short-Term Research Scholar – Genetics, **Yale School of Medicine** June 2017 – September 2017

Principal investigator: **Prof. Daniel C. DiMaio**

- **Investigation of structural patterns of the C-terminus of L2 minor capsid protein from Alpha and Beta-papillomavirus**
  - This work culminated in a manuscript published to *Cell* (doi: 10.1016/j.cell.2018.07.031)

Key technologies: Molecular modeling (PyMol), peptide docking (HADDOCK), coarse-graining (MARTINI), MD simulations (GROMACS, CHARMM), cloning, peptide expression and purification, nuclear magnetic resonance (NMR)

Publications and Patents

In preparation:

- **Silva, G.M.,** Lam, K., Dalgarno, D.C., Rubenstein, B.M. Mutations in a human kinase cause drug resistance by shifting conformational state distributions.
- **Silva, G.M.,** Bloomfield, O., Li, W., Harley, A., Rubenstein, B.M., Patel, J., Ytreberg., M.F. Pep-BARD: A Peptide Binder Dataset Combining Structural and Affinity Data.
- Liu, N., Ho, J., Daru, S., **Silva, G.M.,** Incandela, J., Reda, S., Rosenstein, J., Larkin, J., Rubenstein, B.M. Computing Using Biofilms: Natural XY Machines.

Pre-prints:

- **Silva, G.M.,** Cui, J.Y., Dalgarno, D.C., Lisi. G.P., Rubenstein, B.M. Predicting Relative Populations of Protein Conformations without a Physics Engine Using AlphaFold2. *bioRxiv* doi: 10.1101/2023.07.25.550545 (2023)

Published:

- **Silva, G.M.,** Yang, J., Leang, B., Huang, J., Weinreich, D., Rubenstein, B.M. Covalent docking and molecular dynamics simulations reveal the specificity-shifting mutations Ala237Arg and Ala237Lys in TEM beta-lactamase. *PloS Comp. Bio* (2022)
- Tang, W.S., **Silva, G.M.†,** Yang, J., Kirveslahti, Skeens, E., Feng, B., *et al.* A topological data analytic approach for discovering biophysical signatures in protein dynamics. *PloS Comp. Bio* (2022)
- Keita, H., Santos, C.B.R., Ramos, M.M, (...) **Silva, G.M.** *et al.* Assessment of the hypoglycemic effect of Bixin in alloxan-induced diabetic rats: in vivo and in silico studies. *Journal of Biomolecular Structure and Dynamics (*2021)
- Zhang, P., **Silva, G.M.,** Deatherage, C., Burd, C., DiMaio, D.C. Cell-penetrating Peptide Mediates Intracellular Membrane Passage of Human Papillomavirus L2 Protein to Trigger Retrograde Trafficking. ***Cell*** (2018)
- B.S. Cruz, J.V., Serafim, R.B., **Silva, G.M.** *et al.* Design of new protein kinase 2 inhibitors for the treatment of inflammatory diseases using QSAR, pharmacophore-structure-based virtual screening, and molecular dynamics. *J. of Mol. Modeling (*2018)
- Santos, C.B.R., Ramos, R.R., Ortiz, B.L.S., **Silva, G.M.** *et al.* Oil from the fruits of Pterodon emarginatus Vog.: A traditional anti-inflammatory. Study combining in vivo and in silico. *Journal of Ethnopharmacology (*2018)
- Castillo-Ordóñez, W.O., Tamarozzi, E., **Silva, G.M***. et al.* Exploration of the Acetylcholinesterase Inhibitory Activity of some alkaloids. *Neurochemical Research* (2017)

† Shared first authorship

Patents:

- Provisional **U.S. patent:** Cell-Penetrating Peptides and Methods of Use Thereof, US Patent App. 17/057,501

Selected Presentations

- Oral presentation at the *2023 Biophysical Society Annual Meeting*

"Mutations in a Human Kinase Cause Drug Resistance by Shifting Conformational Landscapes" (San Diego, 2023)

- Poster presentation at the *2022 Biophysical Society Annual Meeting*

"Exploiting Conformational Transitions in Protein Kinases for the Discovery of Polyvalent Inhibitors" (San Francisco 2022)

- Poster presentation at the *2021 Biophysical Society Annual Meeting*

"Covalent Docking and Simulations Reveal the Specificity-Shifting Mutations A237R/K in TEM beta-lactamase" (Remote, 2021)

- Poster presentation at the *2021 MCB Graduate Program Annual Retreat*

"Machines Measuring Molecules: Accelerating Discovery Through Active Learning Docking" (Providence, 2021)

- Oral presentation at the *Yale Global Health Institute Annual Symposium*

"From Chaos to Order: Investigating Structural Patterns in Alpha and Beta-papillomavirus" (New Haven, 2018)

- Poster presentation at the *X-Meeting Bioinformatics International Conference*

"Binding Site Plasticity Analysis of E6 Oncoprotein from High-Risk HPV Variants" (Belo Horizonte, 2017)

Teaching & Mentorship Experience

Computational Biophysics Mentor – Rubenstein Group, Brown University 2022 - Ongoing

Supervisor: Professor Brenda M. Rubenstein

- Scheduled or on-demand meetings with undergraduate and graduate students in the group seeking help with the implementation of biophysical simulation methods or analysis of results
- Preparation and curation of relevant scientific literature, tutorials, external resources, etc.
- Orientation with study design, hypothesis testing and effective result communication
- Undergraduate students mentored: **9 (3** current, **6** past)
- Publications and presentations with mentored students:
    - Silva, G.M**., Lam, K.**, Dalgarno, D.C., Rubenstein, B.M. (manuscript in preparation)
    - Silva, G.M., **Bloomfield, O., Li, W.**, Harley, A., Rubenstein, B.M., *et al.* (manuscript in preparation)
    - **Faith, K., Vemuri, V**., Silva, G.M., D.C., Dalgarno, Rubenstein, B.M., *UTRA Symposium* (2023)
    - Silva, G.M**., Lam, K.**., Dalgarno, D.C, Rubenstein, B.M., *The Biophysical Society Annual Meeting (*2023)
    - Tang, W.S., Silva, G.M., Yang, J., Kirveslahti, **Skeens, E., Feng, B.**, *et al. PloS Comp. Bio* (2022)
    - Silva, G.M., Yang, J., **Leang, B.**, Huang, J., Weinreich, D., Rubenstein, B.M. *PloS Comp. Bio* (2022)

Graduate Teaching Assistant, Virology – MCB, Brown University Fall 2020

Supervisor: Professor Amanda Jamieson

- Weekly office hours and review sessions
- Supervision and mentoring of undergraduate teaching assistants
- Administrative support (grading, preparation of teaching materials and exams, etc.)

Graduate Teaching Assistant, Introduction to Bioinformatics – FMRP, Universidade de São Paulo Fall 2016/Spring 2017

Supervisor: Professor Silvana Giuliatti

- Weekly office hours and lectures
- Administrative support (grading, preparation of teaching materials and exams, etc.)

Tutor, Organic Chemistry I and II – FCFRP, Universidade de São Paulo March 2015 – November 2015

Supervisor: Professor Dionéia Camillo

- Weekly review sessions for students at the Ribeirão Preto School of Pharmaceutical Sciences

Leadership & Organizations

- Member, **Admissions Committee, MCB Graduate Program** – Brown University (2020, 2021)
- Director of International Communications, **OMICS Club** – Universidade de São Paulo (2018)
- Organizer, **Tripartite Bioinformatics Course** – FMRP (2016)
- Co-Founder, **Committee for Science Communication**, Centro Acadêmico Lourenço Roselino – FCFRP (2016)
- Lead surdo (bass drum), **Grêmio Recreativo Escola de Samba Unidus da Dorotéia** – FCFRP (2013 – 2016)

Software Development and Data Sharing

- **Automated Subsampling Parameter Optimization for Conformational Ensemble Predictions** (in development)
    - A suite for automatically detecting the best MSA subsampling parameters for the purpose of predicting changes in the conformational landscapes of proteins in response to mutations with subsampled AlphaFold2
        - https://github.com/GMdSilva/af2_subsampling_opt

- **Relative State Population Prediction with Subsampled AlphaFold2**
    - Raw data, input MSAs, and code for replication of the results shown in the AlphaFold2 subsampling manuscript
        - https://github.com/GMdSilva/rel_state_pop_af2_raw_data

- **Drug Resistance Beta Lactamase Covalent Docking Results**
    - Raw data, PDB models, and analyzed results from the 2021 TEM beta-lactamase CovDock *Plos. Comp. Bio* manuscript
        - https://github.com/GMdSilva/BLactamaseCovDockResults

- **LINE-1 ORF2 Ligand Discovery Results**
    - Raw data, PDB models, useful scripts and notebooks, and analyzed results from a currently unpublished computational search for strong ligands against the ORF2 endonuclease domain from the LINE-1 retrotransposon
        - https://github.com/GMdSilva/L1ORF2LigandDiscovery

- **PeptideBindingDatabase** (in development)
    - Raw data, PDB models, useful scripts, datasets, MD simulation frames, and analyzed results from a currently unpublished database of peptide/protein interactions (with binding energies) curated for maximum diversity.
        - https://github.com/GMdSilva/PeptideBindingDatabase

- **MMOTrader** (in development)
    - A Python program for market monitoring and small-scale algorithmic trading within a classic massively multiplayer online computer game (CipSoft's Tibia). Currently supports statistical arbitrage or trend following strategies.
        - https://github.com/GMdSilva/TibiaTrader