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The Barroso Research lab: biomolecular interactions, computing, and data-driven science to understand and engineer biological and pharmaceutical systems in a global academic partnership

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Abstract Biomolecular interactions, high throughput computing, and data-driven science have been the central research foundations of the Barroso Research laboratory. We have been developing and applying innovative computational technology, offering a rational computational-based approach to the investigation of protein systems, and discovering key disease-related protein mechanisms, therapeutic agents, biomarkers, and proteins for specific applications and their controlled release. Born in 2001 at the School of Pharmaceutical Sciences at Ribeirão Preto with the genes of transdisciplinary and internationalization, the laboratory has always been well integrated with research groups in Europe, the US, and Latin America. Students from different fields and places have been forged in this environment at the crossroads of Structural Bioinformatics, Molecular Biophysics, Biological Physics, Physical Chemistry, Engineering, Medicine, Food, and Pharma. The more than 50 scientific papers published in high-impact journals, book chapters, and conference talks reflect our contributions to expanding knowledge and advancing Bioinformatics as an important tool to understand nature and guide innovations.

Keywords: antibody, biomolecular interactions, complexation, high-performance computing, molecular simulation, pH effects, virus

1 Background

It is well established that the understanding of the molecular world is the fundamental basis to decipher and treat diseases, to design (bio)pharmaceuticals and new functionalized materials, to optimize bioseparation processes, etc [Creighton, 1983; Wrede and Schneider, 1994; Bello and Schwinn, 1996; Bergethon, 1998; Dill, 1999; Ross and Lahann, 2015; Wagoner *et al*., 2016; Bahar *et al*., 2017; Hoarau *et al*., 2017; Lousa *et al*., 2022]. Molecular modeling involves the numerical simulation and statistical mechanical treatment of the biomolecular systems involved in this vast list of biological problems and applications in industry and biomedical sciences [Leach, 1996; Schlick, 2010]. These computational-based approaches yield the desired ability to describe and control the biological systems in terms of such a molecular view [Wrede and Schneider, 1994; Schlick, 2010; Barroso da Silva *et al*., 2020]. With the continuous advance in computer power and the expansion of access to high-performance computer (HPC) centers, the simulation of these systems started to shift from the computational chemistry (CC) [Lipkowitz and Boyd, 2022; Leach, 1996; Thiel and Hummer, 2013] approach (focused on specific molecules and/or one experimental condition) to be placed in a larger-scale context, more fre-

quently referred to as "Structural Bioinformatics" (SB) nowadays [Luscombe *et al*., 2001; Gu and Bourne, 2009; Samish *et al*., 2014]. This is quite evident in the well-known definition of Bioinformatics proposed by Luscombe, Greenbaum, and Gerstein: "(Molecular) Bioinformatics is conceptualizing biology in terms of molecules (in the sense of Physical chemistry) and applying "informatics techniques" (derived from disciplines such as applied maths, computer science, and statistics) to understand and organize the information associated with biological with these molecules, *on a large scale*" [Luscombe *et al*., 2001]. The emphasis "on a large scale" is probably the remarkable difference between SB and CC or the "Computational Biophysics". In SB, this is done by the same system being simulated in several different experimental conditions and/or different systems being investigated and compared. Computational code development, debugging, and testing to investigate molecules are definitely a common part of them. Slightly different views can be found in the literature. For instance, Samish, Bourne, and Najmanovich highlighted that computation has been an integral part of structural biology since its origin [Samish *et al*., 2014]. With this reasoning, they argued that SB "predates other forms of bioinformatics" and was born with Watson and Crick´s 1953 article [Watson and Crick, 1953]. It is, in fact, a cross-road between molecules, biology, biotechnology, physics, chemistry, and computer science [Barroso da Silva *et al*., 2020]. This is probably a pacific interpretation that also indicates its intrinsic interdisciplinary characteristics.

In the past, computer simulations were dedicated to studying simple and complex fluids [Allen and Tildesley, 1989; Gray *et al*., 2011]. This allowed for establishing a solid background that is presently being driven to a broad, challenging, and inherently transdisciplinary arena necessary for biomolecular systems. It is in this context that our laboratory is firmly rooted. The training, knowledge, and experience acquired in developing and applying new computational tools to study simple liquids [Skaf and Barroso da Silva, 1994; Degrève *et al*., 1995; Barroso da Silva *et al*., 1998, 2001b], and electrolyte solutions (the medium where biomolecular systems are typically immersed) [Degrève and Barroso da Silva, 1999b,a; Barroso da Silva, 1999; Degrève and Barroso da Silva, 2000] contributed to the foundation that evolved to rationalize the molecular structures and their interactions determining biomolecular functionality in the aforementioned applications.

Since the beginning of this journey, it was clear that **electrostatic interactions** play an important role in the complex correlation between structure and biomolecular function, and understanding these interactions was necessary for successfully characterizing the biological systems [Barroso da Silva, 1999]. Perutz, the Nobel Laureate, had already published his landmark paper illustrating that electrostatic interactions "dominate many aspects of protein behavior" including protein folding, assembly, stability, and its biological function [Perutz, 1978]. An interesting observation is that it was in this work that Perutz discussed that the assembly of proteins from the tobacco mosaic virus proteins is triggered by electrostatic interactions between neighboring subunits. Therefore, it was well understood at this point that the route to fully understanding complex biological systems as the viruses had to pass through complete knowledge of the electrostatic interactions. The relation between virus evolution and electrostatic features has recently been confirmed for SARS-CoV-2 too [Barroso da Silva *et al*., 2022]. In fact, it is the redox behavior that controls a great number of processes, including enzymatic catalysis, conformational changes, transport mechanisms, and the action of drugs and biopharmaceuticals [Perutz, 1978; Creighton, 1983; Garcia-Moreno, 1995; Barroso da Silva, 1999; Borkovec *et al*., 2001; Barroso da Silva and Dias, 2017; Frigori *et al*., 2020].

2 Overview of the laboratory

The laboratory was established by **Fernando** Luís **Barroso** da Silva as the principal investigator (PI) at the School of Pharmaceutical Sciences at Ribeirão Preto (FCFRP) at the University of São Paulo (USP) in August of 2001 thanks to the financial support of the "Young Investigator Grant in Emerging Research Institution" program from the São Paulo State Research Support Foundation (FAPESP). This internationally competitive grant was a real opportunity for young researchers trained in excellence centers overseas and acting on modern topics to nucleate a new laboratory, particularly in places in the State of São Paulo where their main subjects were not yet covered by others. In those early days, the first group members were: a) one undergraduate student from pharmaceutical sciences – Fabio Figueiredo (R&D director at Procter & Gamble), b) another one from physics – Pedro Autreto (Assistant Professor at UFABC), c) one master's student from molecular biophysics – Sidney Carvalho (Assistant Professor at Unesp), d) and one junior postdoc fellow with a strong background in CC – Sergio M. Vechi (Data scientist at Utilifeed, Sweden). It was the beginning of Computational biophysical-chemistry and Structural Bioinformatics at FCFRP/USP that added these research topics to its consolidated research tradition. At the National Council for Scientific and Technological Development (CNPq), the lab was initially registered under the name "Laboratory of Computational Biophysical Chemistry" (BPC – after the Portuguese acronym, an alias for "Barroso Research lab") to reflect its biophysical and physical-chemical approach in the development of Structural Bioinformatics.

Biomolecular interactions, computing, and data-driven science have been our central research foundations. They are keywords often presented in all our publications. We have been developing and applying innovative computational technology and offering a rational computational-based approach to the investigation of protein systems and to the discovery of key disease-related protein mechanisms, therapeutic agents, biomarkers, ideal food proteins for specific applications and their controlled release (*e.g.* [Teixeira *et al*., 2010; Barroso da Silva, 2013; Barroso da Silva *et al*., 2016; Delboni and Barroso da Silva, 2016; Montellano Duran *et al*., 2018; Smith *et al*., 2020; Poveda-Cuevas *et al*., 2021; Giron *et al*., 2021]) Following Ken Dill´s "metaphor of the pyramid" [Dill, 1999] as the inspiration for our work strategy (see **Figure 1**), we have been researching with three significant aims:

a) Methodological development of computational tools, methods, and simulation software for (bio)molecular systems (*e.g.* the "**S**tatistical **R**everse **M**onte **C**arlo" (SRMC) [Barroso da Silva *et al*., 1998, 1999, 2007], the "**F**ast **p**roton **t**itration **s**cheme" (FPTS) for proteins [Barroso da Silva and MacKernan, 2017; Barroso da Silva, 2024] and nucleic acids [Barroso da Silva *et al*., 2017], the "**Pro**tein-protein complexes by **m**acroscopic **e**lectrostatic **the**ories and **u**ser-friendly **s**imulations" (PROMETHEUS) portal (`http://glu.fcfrp.usp.br:8180/prometheus/`) [Calixto, 2010], the "**Mol**ecular **S**tructure **A**nalysis" (MOLESA) portal (`http://glu.fcfrp.usp.br:8180/molesa/`) [Calixto, 2010], OPEP6 – the New Constant-pH Molecular Dynamics Simulation Scheme with OPEP Coarse-Grained Force Field [Barroso da Silva *et al*., 2019; Barroso da Silva, 2024], the "**PR**ediction **O**f ele**C**trostatic **E**pitopes bas**ED** on **pKa** shifts" (PROCEEDpKa) [Poveda-Cuevas *et al*., 2020], and the "**F**ast c**O**arse-grained p**R**otein-pro**T**ein mod**E**l" (FORTE) [Neamtu *et al*., 2022a,b; Barroso da Silva, 2024]);
b) Investigations of generic aspects of ionic strength and proton (pH) effects in the stability, structure, and function of biological macromolecules in solution, and;
c) Application of this technology to functionally interesting systems, for example, in viral and food proteins, immune response, antibody development and optimization, biomaterials, calcium-binding, and protein-RNA interactions. Together with in-house developed methods and computer codes, to achieve our goals, we complement our toolboxes with a diversity of others available in the literature [Barroso da Silva *et al*., 2020]. These aspects cooperatively provide essential tools and views to integrate and provide a molecular basis for the challenging of our long-range goals.

3 Research topics: basic and applied science together via computer simulations

When in 2003 Anna Tramontano published her book entitled "The Ten Most Wanted Solutions in Protein Bioinformatics" [Tramontano, 2005], her views reinforced that the laboratory was on the proper track to address important and challenging scientific problems. Several of our main research topics were listed in her top ten problems: a) "Predicting Protein Features from the Sequence"; b) "Protein Structure Prediction" (PSP); c) "Protein-Protein Interaction" (PPI); d) "Protein Design" (PD); and e) "Protein Engineering" (PE). In the beginning, before working with these "hot topics", the lab invested in expanding the base of Dill´s research pyramid [Dill, 1999] as illustrated in **Figure 1**.

Simpler molecular systems (calcium binding to proteins [Barroso da Silva *et al*., 2001a, 2005; de Carvalho *et al*., 2006, 2008] and colloidal systems [Barroso da Silva *et al*., 2002]) were investigated by us using in-house written computer simulation codes. From these systems, we moved up words in Dill´s pyramid taking classes of problems: i) evolutionary algorithms approach for the PSP [de Lima *et al*., 2009; Ishivatari *et al*., 2011; Brasil *et al*., 2013], and ii) studies of the protein-polyelectrolytes complexation (*e.g.* [Autreto *et al*., 2003; Barroso da Silva *et al*., 2006; Barroso Da Silva and Jönsson, 2009; Prudkin-Silva *et al*., 2020]). While the first topic is of broad interest and is presently under strong evidence due to the impressive achievements of AlphaFold2 [Jumper *et al*., 2021], the second one is of great interest in more specific areas related to food, brewing, pharma, and bioprocess technology.

Protein-polyelectrolyte interactions: from applications to a "toy model" to understand complexation phenomena

The study of protein-polyelectrolyte interactions is important not only because of its applications, such as the encapsulation of active ingredients for food and medicinal drugs and their controlled release, and stabilization of food emulsions but also due to its potential to enhance our understanding of fundamental chemical and physical phenomena [Doublier *et al*., 2000; Whitaker *et al*., 2002; de Kruif *et al*., 2004; de Vries and Cohen Stuart, 2006; Bromberg, 2008; Srivastava *et al*., 2017; Eghbal and Choudhary, 2018]). It was an interesting and useful system to be explored to allow the understanding of new peculiar effects observed in mixtures involving charged macromolecules [Kirkwood and Shumaker, 1952; Jönsson *et al*., 2007]. This system was also a good prototype for PPIs. Several experiments have shown the complexation between like-charged biomolecules, a phenomenon that is often cited as the "complexation on the wrong side of the isoelectric point" [Grymonpré *et al*., 2001; de Kruif *et al*., 2004; Wittemann and Ballauff, 2006]. At a first glance, this behavior contradicts the basic laws of physics. Invoking a simplified colloidal-like molecular description of the system, Linse, Stoll, de Vries, and collaborators carried out computer simulations to explain the mechanism [Carlsson *et al*., 2001, 2003; De Vries, 2004; de Vries and Cohen Stuart, 2006]. Yet, pH effects were not completely incorporated into the first models despite being well-known to be a critical parameter in chemical and biological systems – see a detailed discussion in ref. [Barroso da Silva and Dias, 2017].

The development of a constant-pH simulation method to model this system allowed us to explain the experimental behavior in a pure electrostatic context [Barroso da Silva *et al*., 2006; Barroso Da Silva and Jönsson, 2009; Barroso da Silva, 2013; Srivastava *et al*., 2017; Montellano Duran *et al*., 2018; Prudkin-Silva *et al*., 2020; Lunkad *et al*., 2022]. pH-Responsive biomolecules are especially affected by such peculiar physical interactions. In parallel, the larger energetic barriers of the systems offered an opportunity to build out new computational strategies for the proper sampling of such systems [Barroso da Silva *et al*., 2006]. It is worth noting that the simulation of these systems is quite demanding in terms of CPU time. A typical complexation simulation run for a relatively small protein such as alpha-lactalbumin and short polyelectrolyte chain with 21 monomers requires ca. 54h in a Linux box with an Intel Xeon E5-2690v4 processor for each physical-chemical condition [Barroso da Silva *et al*., 2006]. Often, at least three replicates are necessary to estimate statistical errors per case and run with different pH, salt concentration, and mutation conditions. Even for these

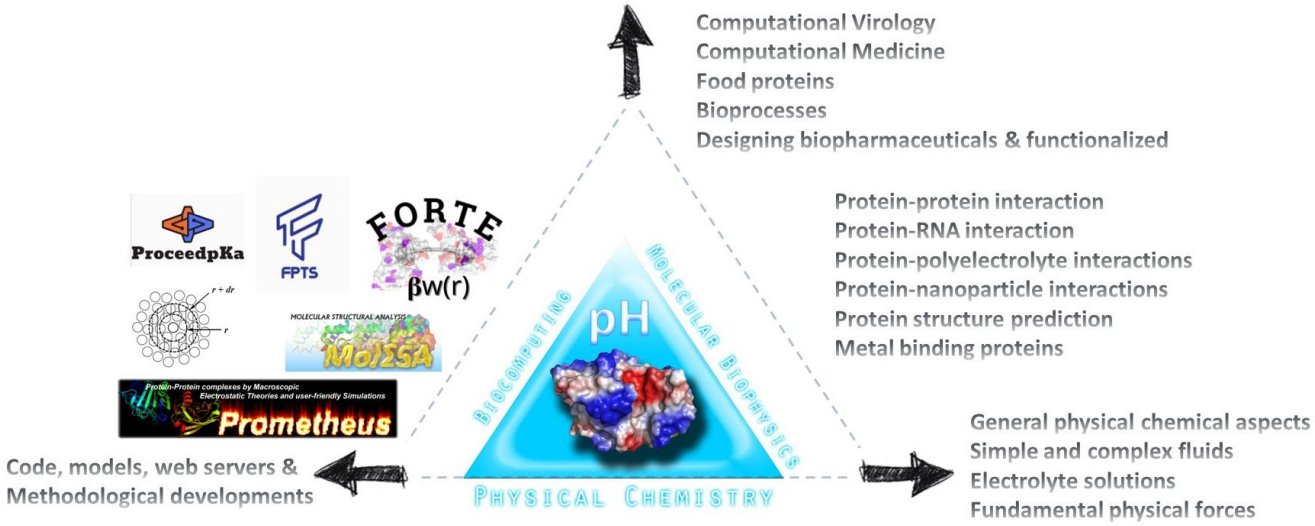

Figure 1. Structural Bioinformatics with a biophysical and physical chemistry strong accent. This schematic view is based on Dill´s research pyramid adapted to our work strategy. As broad as the bottom is, more challenging achievements can be conquered to advance biological, agricultural, pharmaceutical, and biomedical fields. See the text for details.

systems, it is easy to see that these simulations well fit into the so-called "High-throughput computing" [Buyya *et al*., 2013], the "large-scale context" of SB, and consequently the strong dependence on HPC centers for successful projects. This is schematically shown in **Figure 2**. Note the difference in the number of runs/simulated conditions between CQ and SB, and the possibility to produce a databank from the huge amount of output data from SB that can be used, for example, to extract new physical insights by applying machine and deep learning techniques.

The physical mechanisms found in the computation of this system were also described for protein-nanoparticle ones [Barroso da Silva *et al*., 2014]. After the development of a new titration computer model for RNA [Barroso da Silva *et al*., 2017], this project has also been extended to protein-RNA interactions where the p19 viral protein was studied [Barroso da Silva *et al*., 2018]. Therefore, we could contribute to the general physicochemical understanding of the complexation phenomena, the life cycle of tombusviruses (p19 protein is known as a significant virulence factor [Hull, 2014]), and the extensive role of RNA in the cell's life.

From protein-protein interactions to new materials, and Computational Virology and Immunology

Proteins are macromolecules that almost always need another biomolecule to interact with and undertake biological functions. This makes the PPI a central topic in biological systems. Metabolism, growth, immune response, and essentially all cellular activities are a result of PPIs [Braun and Gingras, 2012; Miernyk and Thelen, 2008; De Las Rivas and Fontanillo, 2010]. Industrial applications are also dependent on the PPIs [Delboni and Barroso da Silva, 2016; Alrosan *et al*., 2021]. Several computational tools are available for the analysis of PPI [Kotlyar *et al*., 2019; Tuncbag *et al*., 2009; Zacharias, 2005; Neamtu *et al*., 2022a,b]. However, rarely pH and salt effects are incorporated into the predictive computational tools, a clear drawback also in the recent machine and deep learning works. For example, AlphaFold2 is based and consequently biased on available structural data produced obtained in a specific experimental condition of pH and salt [Jumper *et al*., 2021; Bryant *et al*., 2022]. This is one of the gaps that we mostly focused on in our works in this field [Barroso da Silva and Dias, 2017; Neamtu *et al*., 2022a,b]. Ongoing studies in collaboration with Prof. Derreumaux´s lab are trying to refine the previously developed constant-pH simulation models to improve their power (both in terms of accuracy, size of the systems, and convergence features) for studying molecular mechanisms that govern a wide variety of important biological processes [Pasquali *et al*., 2019; Barroso da Silva *et al*., 2019]. This is the main open question needed in terms of computational developments that remains a great challenge [Barroso da Silva and MacKernan, 2017; Barroso da Silva *et al*., 2020; Barroso da Silva, 2024].

Concerning PPI, studied systems include a diversity of examples from food proteins to spidroins and septins [Autreto *et al*., 2003; Jönsson *et al*., 2007; Delboni and Barroso da Silva, 2016; Barroso da Silva *et al*., 2016; Mendonça *et al*., 2019]. However, as mentioned above, viral proteins seem to have a special relationship with electrostatic interactions [Ramaraj *et al*., 2012; Kiyoshi *et al*., 2014; Song *et al*., 2016]. This motivated us to apply our knowledge of biomolecular interactions in virology, taking the benefits of a laboratory build-up at the interface between biophysics, biological physics, bioinformatics, physical chemistry, biology, and pharmaceutical and medical sciences. Infectious diseases are per se a transdisciplinary field [Lousa *et al*., 2022]. Moreover, protein-protein (PP) interfaces of viruses are richer in titratable groups in comparison with other PP interfaces [Ramaraj *et al*., 2012]. This makes them even more appealing for electrostatic studies.

In terms of molecular science, a virus can be reduced to

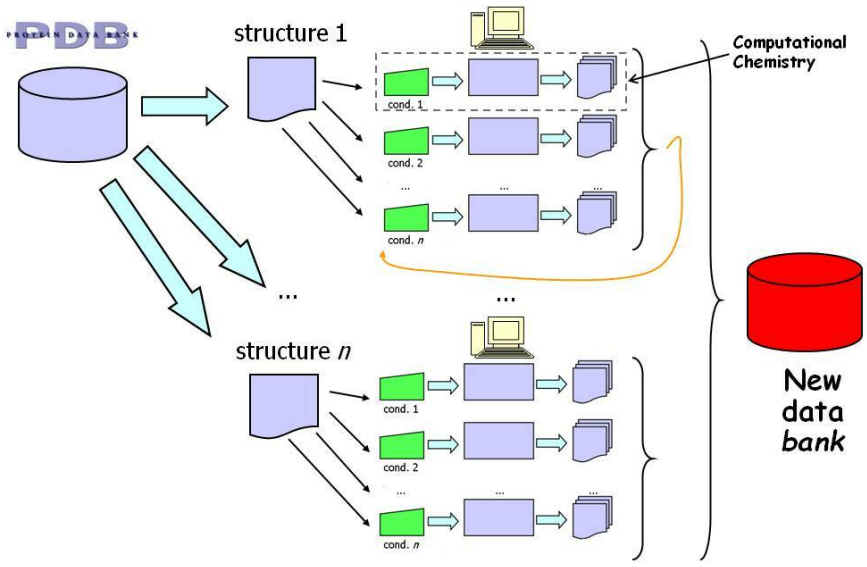

Figure 2. A scheme to illustrate the set of simulation runs involved in a typical project in Structural Bioinformatics. It can combine several different input structures (or mutations) and experimental physical-chemical conditions (*e.g.* pH, ionic strength, protein concentration). Initial structures can be both from the experiments (available, for instance, at the Research Collaboratory for Structural Bioinformatics Protein Data Bank) and predicted also by computational methods.

a set of proteins interacting with different biological partners: a) self-assembly to form the viral capsid, b) host-proteins (*i.e.* the host-pathogen interactions for the infectivity/virus life cycle or the antigen-antibody for the immune response), c) therapeutical binders, which include drugs in general and biopharmaceuticals, d) nucleic acids and e) lipids [Modrow *et al*., 2013]. The physical-chemical properties of these molecules, such as their electric charge, dipole moment, molecular mass, and medium (pH, ionic strength, temperature) affect their stability, their biomolecular interactions, and their interplay determining the viral tropism, transmission, and pathogenicity [Poveda-Cuevas *et al*., 2018]. Among interaction processes, antibody-antigen recognition is an intensive field since immunology reactions are of major importance in health care. Specificity for antibody-antigen interactions is a topic that can be even much more complex (see, for instance, refs. [Regenmortel, 2014; O'Kennedy *et al*., 2017; Dobaño *et al*., 2021]) and has been receiving our attention. Here is a good example of an open question from the application side that has a close relationship to needed modeling and computational efforts.

Discussions with Profs. Marek Cieplak (Polish Academy of Science) and Rudi Podgornik (Chinese Academy of Sciences) stimulated the lab to apply its knowledge to viruses. We began by studying the self-assembly of capsid proteins of norovirus in collaboration with Drs. Bressanelli and Boulard's team at Univ. Paris-Saclay, as reported in [Carvaillo *et al*., 2024]. Subsequently, we shifted our focus to characterizing the molecular differences between strains of Zika virus [Poveda-Cuevas *et al*., 2018]. Electrostatic features and dynamical properties were investigated to shed light on the molecular reasons for their differential virulences [Poveda-Cuevas *et al*., 2021, 2020, 2022; Barroso da Silva *et al*., 2022; Poveda-Cuevas *et al*., 2023]. These works opened up new routes to investigate infectivity and how to block it. Such knowledge was ready to be repurposed for coronaviruses when SARS-Cov-2 arrived. In the early days of the COVID-19 pandemic, our computer simulations predicted that the receptor-binding domain (RBD) of SARS-CoV-2 could bind to the cell receptor Angiotensin-Converting Enzyme-2 (ACE2) [Giron *et al*., 2020]. This finding was instrumental in corroborating the groundwork for the creation of anti-SARS-CoV-2 vaccines with a focus on the RBD [Prates-Syed *et al*., 2021]. Additionally, our research demonstrated that the RBD could potentially attach to the monoclonal antibody (mAbs) CR3022, which had been previously sourced from SARS-CoV-1 plasma obtained from a recovered patient [Giron *et al*., 2020]. Subsequently, these theoretical predictions were substantiated by laboratory experimentation [Walls *et al*., 2020]. In subsequent manuscripts, this work was extended to the design of peptides for the production of vaccines and to the investigation of other variants including Omicron/BA.1, BA.2, and BA.3 and 32 mAbs that were recently discovered [Giron *et al*., 2021; Prates-Syed *et al*., 2021; Giron *et al*., 2022; Neamtu *et al*., 2022a,b]. We addressed key molecular mechanisms of both the host-pathogen and the antigen-antibody interactions for SARS-CoV-2 wildtype and its variants. ACE2 polymorphism was investigated revealing that two ACE2 mutations typically found in Europeans increase the RBD-ACE2 binding affinities suggesting increased risky susceptibility for this population [Giron *et al*., 2022]. To our knowledge, no previous studies have investigated and compared the RBD-ACE2 and RBD-mAbs binding affinities for such a large number of cases under the same conditions and using a common computational-based approach for all of them at the same time. This is an ideal example of the "large-scale" idea of SB. Such information contributes to the understanding of the virus evolution, the molecular aspects of the physiopathology of COVID-19, and future directions to be explored for the de-

velopment of new therapeutical options to treat coronavirus patients.

A new computational approach towards an optimal mAb with higher binding affinity to the RBD of SARS-CoV-2 spike proteins from different variants was proposed as a fruit of the collaboration with the group led by Prof. Laaksonen (SU) [Neamtu *et al*., 2022a,b]. We could find a newly engineered mAb with a higher affinity for the RBD domain that could prevent its interaction with ACE2. This new mAb was found ideal for both Delta, Omicron/BA.1 and Omicron/BA.2 variants [Neamtu *et al*., 2022a]. This offers new routes for the treatment of many other diseases. Antibodies have many applications in health. Examples can be found in Alzheimer's disease, cancer, genetic disorders, metabolic diseases, etc [Berger *et al*., 2002; Lu *et al*., 2020; Neamtu *et al*., 2022a; Abbott, 2022]. Efforts are already being made to optimize binders for flaviviruses in collaboration with Dr. Flamand´s team from the Pasteur Institute in Paris. This corresponds to the PD and PE problems listed by Tramontano [Tramontano, 2005]. It is our current and open challenge. Taking together, from the nomenclature point of view, all these efforts expand SB to overlap with computational virology, immunoinformatics, and computational medicine.

4 Transdisciplinary and internationalization

Transdisciplinary and internationalization are in the genes of the lab. The laboratory was born out well integrated with research groups in Sweden (Department of Theoretical Chemistry, Lund University – the *alma mater* of Prof. Barroso), Venezuela (Prof. Olivares-Rivas´s, Department of Chemistry, Universidad de Los Andes), and their academic networks. A joint effort from experts coming from complementary research areas, different cultures, and regions of the world is the most effective way to provide a deeper understanding of challenging scientific problems. Aware of it, besides national co-authors from different nearby institutions [Computer Science and Mathematics Institute/USP; the Department of Physics, the Department of Chemistry, and the Department of Mathematics at the Faculty of Philosophy, Sciences, and Letters at Ribeirão Preto (FFCLRP/USP); the Department of Physics and the Department of Chemistry at School of the Sciences/State University of São Paulo; School of the Medicine/Federal University of Triâangulo Mineiro (UFTM); Institute of Biomedical Sciences/USP; São Carlos Institute of Physics/USP], it became easy to say that the sun is always at its meridian concerning where our collaborators are. Collaborations have been distributed over the Earth including new partners from other research groups in **Sweden** [Arrhenius Laboratory, Stockholm University (SU); Department of Engineering Sciences and Mathematics, Luleå University of Technology; Department of Materials Science and Engineering, Royal Institute of Technology (KTH); the Nordic Institute for Theoretical Physics/Nordita] and other countries: **Argentina** [Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales; Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR)], China (State Key Laboratory of Materials-Oriented and Chemical Engineering, Nanjing Tech University), the Czech Republic (Department of Physical and Macromolecular Chemistry, Charles University), France (Institut National de la Transfusion Sanguine; Institut National de la Santé et de la Recherche Médicale, UMR_S 1134; INSERM/Dynamique des Structures et des Interactions Moléculaires; Université de Paris; Laboratoire de Biochimie Theórique, UPR 9080/CNRS, Institut de Biologie Physico Chimique; Structural Virology/UMR 3569, Institut Pasteur; Institute for Integrative Biology of the Cell, Université Paris-Saclay), **Germany** (Institute for Computational Biomedicine/Jülich), **Ireland** (UCD Institute for Discovery; UCD School of Physics, University College Dublin and CECAM – Centre Européen de Calcul Atomique et Moléculaire/Irish node), **Italy** (Department of Chemical and Geological Sciences, University of Cagliari), **Portugal** (Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa), **Romania** ("Grigore T. Popa" University of Medicine and Pharmacy of Iasi; Centre of Advanced Research in Bionanoconjugates and Biopolymers), the **US** [Department of Chemical and Biomolecular Engineering and the Department of Food, Bioprocessing, & Nutrition Sciences, North Carolina State University (NCSU); School of Medicine, The University of Pennsylvania], **Norway** [Centre for Materials Science and Nanotechnology, University of Oslo (UO)], and Vietnam (Faculty of Pharmacy, Ton Duc Thang University).

A long-range collaboration is strongly dependent on continuum funding. Thanks to the financial support from different national (USP, Fapesp, CNPq, and Coordination of Superior Level Staff Improvement [Capes]) and international organizations [University Global Program Network (UGPN), UCD Seed program/Ireland, The Swedish Foundation for International Cooperation in Research and Higher Education (Stint), Swedish National Infrastructure for Computing (SNIC), USPC-USP Joint Research Projects/France, USPC-USP Joint Pedagogical Projects/France, Guest Professor programs from Université Paris Cité France], it was possible to nucleate additional networks and to establish stronger partnerships both in research and in teaching activities. More than the synergistic contribution of these collaborations, scientific papers published together, and students' training (see below some initiatives), they strengthen the bonds of friendships.

The STAMiNA Global Network

Within the framework of the UGPN and Stint/Sweden, the STAMiNA (**STA**tistical **M**echanics **i**n **NA**nobiotechnology) Global Network (`http://cthulhu.che.ncsu.edu/~erik/STAMiNA/STAMINA_People.html`) was established in 2013 as an international community of researchers in the areas of Statistical Mechanics, Nanotechnology, Biotechnology, Thermodynamics, Computer-Based Materials Design, Bioinformatics, and Nanobiotechnology. The initiative was proposed by Prof. Erik E. Santiso (NCSU), Prof. Fernando Barroso da Silva (FCFRP), Prof. Mathias Boström (KTH and UO), Prof. Keith E. Gubbins (NCSU), Prof. Aatto Laaksonen (SU), and Prof. Clas Persson (UO). Both Profs. Boström and Persson were key people in the initial funding

support via the Stint award.

The main goal of STAMiNA is to serve as a virtual platform for scientific collaboration between researchers in different countries interested in statistical mechanics application in bionanotechnology. The group seeks to advance the understanding of molecular interactions between biomolecules and biomimetic materials, as well developing new (bio)materials that exploit this understanding. This object is pursued by: a) identifying common research interests among our members; b) sharing knowledge electronically and by organizing regular international workshops; c) exchanging students and postdoctoral researchers between our groups to further collaborate and learn about each other's areas of expertise; and d) pursuing the publication of scholarly works to disseminate the results of our collaborative research.

The Santiso lab, at NCSU, has collaborated extensively with the BPC. Our joint activities include the development of molecular models for biomimetic polymers, biomineralization, the effect of polyelectrolyte interactions with whey proteins [Srivastava *et al*., 2017]], and more recently the modeling of food-related molecules [Barroso da Silva *et al*., 2020]. Prof. Santiso's expertise in force field development (*e.g.* [Weiser and Santiso, 2019; Chadwick *et al*., 2019; Shi *et al*., 2019; Walker *et al*., 2020]), the atomistic modeling of activated processes [Santiso, 2014], and the fitting of coarse-grained polymer models [Clark, 2021], provides a synergistic and complementary set of skills to those of the BPC. In addition to these scientific collaborations, professors Barroso da Silva and Santiso have co-organized several workshops on molecular modeling, including a workshop on Molecular Interactions and Nanobiological Applications at the USP in Ribeirão Preto, Brazil (June 23-25, 2013), and at KTH, Sweden (January 13-14, 2014), as part of a project funded by the UGPN. The KTH event was organized in collaboration with an important member and co-creator of this network, Prof. Boström, who graciously hosted the entire team at KTH. Both Profs. Santiso and Barroso have also co-organized two other workshops funded by CECAM and together with Dr. Donal MacKernan (UCD), one entitled "Controlling Food Protein Folding and Aggregation: Challenges and Perspectives in Industry, Experiments and Simulation", on Aug 17-19, 2016 in Dublin, Ireland, and one entitled "Simulation of open systems in Chemistry, Pharma, Food Science, and Immuno-diagnostics: Rare-event methods at constant chemical potentials including constant pH – an E-CAM Industry Scoping Work", in 2021-2022 in Dublin, Ireland. These interactions have served both groups to interact not only with other academics but also with industry stakeholders interested in the application of molecular modeling techniques to their respective applications. In particular, the latter workshop addresses a major issue at the forefront of molecular modeling of complex systems and is an example of the synergy between both groups, combining methods to study activated processes with methods to maintain constant chemical potentials.

The UPC-USP International Laboratory in Structural Bioinformatics

Université Paris Cité (UPC) and USP are increasing their efforts to establish long-term collaborations in strategic research fields. Combining their complementary expertise and common interest in SB and biomedicine, the bioinformatics team in Inserm unit UMR-S1134 (former UMR-S665), Dynamics of Structure and Interactions of Macromolecules in Biology (DSIMB), led at that time by Prof. Catherine Etchebest (UPC), associate with BPC to establish a joint International Laboratory in SB in 2015. The aim is to expand the synergistic collaboration across the applications of SB through the scientific borders of virology, biomolecular engineering, biochemistry, biophysics, and theoretical/computational chemistry. The partnership involves not only research but also teaching, training, and the promotion of the field to the general public. Joint graduated disciplines such as "Structural Bioinformatics topics in health" were offered by the two PIs in both institutions. The full co-supervision of Ph.D. students ("cotutelle" in French) is a key component of this fruitful collaboration. The next step now is the implementation of a broader double-degree program. All these initiatives also help to integrate the two labs into different research networks promoting a global partnership to solve the most challenging molecular science problems.

DSIMB is composed of more than 10 permanent members located in Paris but also on Reunion Island. Besides recognized expertise in the development of methods for predicting the 3D structure of proteins or protein complexes [Melarkode Vattekatte *et al*., 2020; Maljković *et al*., 2022; Fogha *et al*., 2021; Krisko and Etchebest, 2007; Cretin *et al*., 2021; Bornot *et al*., 2010], DSIMB has acquired an international reputation in the study of membrane proteins [Rao *et al*., 2020; de Brevern *et al*., 2005; Hinsen *et al*., 2015; Vaitinadapoule and Etchebest, 2017; de Brevern *et al*., 2009; Lacapère *et al*., 2007; Tèletchèa *et al*., 2019; Phan *et al*., 2010; Postic *et al*., 2017; Etchebest and Debret, 2010; Barneaud-Rocca *et al*., 2013; Galochkina *et al*., 2019], a topic that was at the origin of the fruitful BPC–DSIMB collaboration. Indeed, molecular dynamics simulations performed by DSIMB on aquaporin, a well-known and important membrane protein, were revealing an intriguing behavior that led to thinking of the role of pH. BPC was the ideal partner to address this question. Since then, DSIMB has enlarged its research topics towards the exploration of nanobodies, which share common characteristics with antibodies. Hence, strengthened by the knowledge acquired in this field, DSIMB and BPC are now in an excellent position to develop computational-based tools for designing new antibodies. This work benefits from a valuable collaboration with the Pasteur Institute in Paris. Moreover, it has got financial support from an international research program between the National Research Agency in France and FAPESP.

5 Human resources: Investing in the future

The lab has been involved in the academic training of students from all levels, from elementary school to postdoc fellows. For instance, to awaken interest in science and this universe of dormant possibilities, the lab contributed to the various programs for elementary and medium school students of the "House of Science", hosted by the "Center of cell-based therapy", a Fapesp excellence center at Hemocentro/Ribeirão Preto. Some activities were carried out together with the "Laboratory for Biological Information Processing" led by Prof. Ricardo Vêncio from the FFCLRP/USP. The lab participates in the internship program for the major in biological sciences program from the Ribeirão Preto Medical School (FMRP/USP). More than 15 students were supervised in these enterprises.

Graduated students from abroad have also been receiving training in the lab for specific projects: Deepti Srivastava (NSCU/USA), Katie Acken (NSCU/USA), Laura Weiser (NSCU/USA), Liangliang "Paul" Huang (NSCU/USA), Natalia M. Duran (UNR/Argentina), and Thomas Fabiani (NSCU/USA). Full remote training was also done even before the COVID-19 pandemic within the French collaborations and the Master's students Gilles Lamothe (UPC) and Hélène Borges (UPC). At present, the team is formed by a) one undergraduate student – Carolina C. Giron (medical student, UFTM), b) two Ph.D. students in Bioinformatics – Ilyas Grandguillaume (BPC/USP and DSIMB/UPC) and Rauni Borges Marques (USP), c) and a recent formed Ph.D. in Bioinformatics – Sergio Alejandro Poveda Cuevas (USP). All of them are researching virus-related problems. The Ph.D. students are supervised by both Fernando Barroso and Catherine Etchebest while Carolina Giron´s project is involved in a partnership with Prof. Aatto Laaksonen (SU).

Besides the local initiatives, the lab regularly runs advanced schools to offer training to bring students to the research level in this transdisciplinary field. Most of these events are organized together with Dr. Ralf Eichhorn (Nordita/Sweden). The APS & ICTP-SAIFR Young Physicists Forum on Biological Physics (2020) is a good example of such events. In fact, Biological Physics is a rapidly developing research area, attracting and challenging researchers from physical, mathematical, biological sciences, and bioinformatics. This offers an ideal scenario to cross-pollinate all these disciplines.

6 Final Remarks

The Barroso Research laboratory is where Structural Bioinformatics, Molecular Biophysics, Biological Physics, Physical Chemistry, Engineering, Medicine, Food, and Pharma meet and merge. Multi-scale and multi-approach modeling of chemical and biological systems are contributing to advancing our deeper understanding of nature. Computer simulations applied to a large-scale scenario of molecular systems offer a great and unique opportunity to review the comprehension of material sciences, biology, and medicine. Investing in the development of molecular models that capture the essential features of real systems, new methodologies, and computer simulation codes has been a promising route to perform cutting-edge research and guide innovations. Partnership with experts from worldwide excellence centers maintains the laboratory integrated with global networks and catalyzes achievements.

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Authors' Contributions

FLBDS is the main contributor and writer of this manuscript. CE and EES were also writers of this manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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