Contact Information

Full name	Alexandre Cassago
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Professional Information

Jan 2023 - Present	**Cryo-electron microscopy specialist**
Professional address	Stanford-SLAC Cryo-EM Center - S^2C^2 SLAC National Accelerator Laboratory - CA, United States URL home page: s2c2.slac.stanford.edu
Sep 2013 - Jan 2023	**Cryo-electron microscopy specialist**
Professional address	Brazilian Nanotechnology National Laboratory - LNNano Brazilian Center for Research in Energy and Materials - SP, Brazil URL home page: lnnano.cnpem.br

Expertise

Experience with different samples: Protein Complexes; Viruses; Nucleic Acids - Protein Interaction; Cell Organelles; Cubosomes; Liposomes; Nanoemulsion; Cellulose Nanocrystals and Fibers.
Experience with SPA: high experience data collection using Falcon 3EC, Falcon 4i and K3 detectors and Selectris X and BioQuantum energy filters. Basic level in Data Processing for SPA.
Experience with Tomography and Subtomography: basic level using Tomography Thermo Fisher software.
Experience with microED: basic level using microED Thermo Fisher software.
Training, assisting, and overseeing users: training users with different skill levels, from beginners to advanced at sample preparation, microscope operation, data collection, and data processing;
Support researcher projects: oversee, mentor and interact with researchers developing strategies for sample preparation, data acquisition, and images analysis;
Strong interpersonal communication skills: ability to work with people from different places and cultures developed over years of work at the microscopy facility;
Workshop and Short-term training: organization of workshops and short-term courses on sample preparation, microscope operation, data processing, and dissemination of the Cryo-EM technique in booth at the main congresses and meetings in the field;
Day-to-day operation: well organized, focused, efficient multi-tasker with strong time management skills. Performing full alignment on cryo-TEM, maintenance, and solving troubleshoot issues together with fields services engineers of Thermo Fisher (Titan Krios G3i, Talos Arctica G2, Talos F200C) and Jeol (JEM-1400Plus) microscopes.

Research Experience

My research experience with electron microscopy started during my first Post-Ph.D. studying the **Glutaminases** - mammalian proteins involved in energy supply of tumor cells. In this study published in **JBC** - 2013 (DOI: 10.1074/jbc.M113.501346) by Negative Stain, showed a direct correlation between the tendency to self-assemble from tetramer oligomerization to long filaments and the activity levels of the three mammalian glutaminases isozymes as previous published by me in **PNAS** - 2012 (DOI: 10.1073/pnas.1112495109). A structural determination by cryo-EM combined with cryo-ET was published in **Nature Structural & Molecular Biology** - 2023 (DOI: 10.1038/s41594-023-01118-0) proposing an allosteric mechanism of inorganic phosphate (Pi)-induced to long filaments of glutaminases and its impact protecting mitochondria from mitophagy.

Structural studies by SPA were carried out in the following years after being hired as Cryo-EM Specialist to support the implementation of the Cryo-EM technique in Brazil at the Cryo-EM Brazilian Nanotechnology National Laboratory (LNNano) the first Cryo-EM Facility in South America. I had the opportunity to contribute to several projects and improving my skills working together with different groups of researchers and subjects. The **Type IV secretion system core complex** - a membrane protein/DNA secretion system from phytobacteria, published in **Nature Microbiology - 2018** (DOI: 10.1038/s41564-018-0262-z) took an apprenticeship through numerous difficulties working with bacterial periplasm proteins, detergents and adsorption of particles on the carbon surface of microscopy grids. The **Septins** - mammalian proteins involved in intracellular processes and cytoskeleton, published in **Cytoskeleton - 2019** (DOI: 10.1002/cm.21569), reviewed the correct assembly for the canonical combination of septins 2-6-7, putting an end to the incorrect order widely disseminated by numerous citations. And the **Mayaro virus**, an emerging arbovirus of the Americas that causes a debilitating arthritogenic disease, published in **Nature Communications - 2021** (DOI: 10.1038/s41467-021-23400-9), highlighting the N-glycosylation sites in the E1-E2 spikes, the proteins responsible for the virus to penetrate host cells and cause infection, a research fully developed in-house about a subject of Brazilian national importance.

Morphological studies of soft matter such as liposomes, cubosomes, and cellulose have also been developed over time, increasing my abilities in the preparation and analysis of different types of samples. **Liposome** widely investigated by the pharmaceutical industry for application in cosmetics and drug delivery systems. The publication at **Langmouir - 2015** (DOI: 10.1021/la5045865) and **Colloids and Surfaces A - 2018** (DOI: 10.1016/j.colsurfa.2018.06.073) are examples of the association between liposomes and hyaluronic acid (HA), a biopolymer that occurs naturally in living organisms, and liposomes as a safe and effective delivery system for small interfering RNA (siRNA). **Nanocellulose**, glucose polymers arranged in either crystalline form or long fiber widely used as coatings and gels, as well as sensing and optoelectronic devices. The publication in **Cellulose - 2020** (DOI: 10.1007/s10570-020-03116-7) suggests a protocol for preparing cellulose samples for transmission electron microscopy that greatly improved the quality of images obtained by this technique, and necessary for the morphology characterization.

Early 2023, I have joined the experienced team from Stanford-SLAC Cryo-EM Center - S^2C^2, one of the three National cryo-EM service centers funded by NIH, located at SLAC National Accelerator Laboratory to continue developing my skills and spreading the cryo-em technique by supporting training, assisting and overseeing user and researcher across the U.S. and abroad.

Academic Education

Sep 2012 - Aug 2013 **Post-Ph.D.**
Brazilian Nanotechnology National Laboratory, LNNano
Brazilian Center for Research in Energy and Materials, CNPEM, Brazil
Title of study: Cryo-EM: visualizing biological macromolecules in different conformational states
Advisor: Dr. Rodrigo Villares Portugal

Sep 2010 - Aug 2012 **Post-Ph.D.**
Brazilian Biosciences National Laboratory, LNBio
Brazilian Center for Research in Energy and Materials, CNPEM, Brazil
Title of study: Structural Studies of Kidney Type Isoform Glutaminase and search for interaction partners
Advisor: Dr. Sandra Martha Gomes Dias

Mar 2006 - Aug 2010 **Ph.D.**
São Carlos Institute of Physics, IFSC
University of São Paulo, USP, Brazil
Title of study: Structural Studies of Selenocysteine Synthase (SELA) from *Escherichia coli*
Advisor: Dr. Otavio Henrique Thiemann

Feb 2003 - May 2005 **M.Sc.**
São Carlos Institute of Physics, IFSC
Federal University of São Carlos, UFSCar, Brazil
Title of study: Molecular Studies of Selenocysteine Synthase (SELA) from *Escherichia coli*
Advisor: Dr. Otavio Henrique Thiemann

Aug 1999 - Fev 2003 **Bachelor of Sciences**
Graduate in Biological Sciences
Federal University of São Carlos, UFSCar, Brazil
Title of study: Intracellular Analysis of DCRA e DCRB Proteins
Advisor: Dr. Flávio Henrique da Silva

Awards

2008 Winner of the 18th Annual Users Meeting from the National Synchrotron Light Laboratory Poster Session, LNLS - Brazil
Title of study: Structural Studies of Selenocysteine Synthase (SELA) from *Escherichia coli*.

2002 Winner of the 2nd National Congress of Scientific Initiation Competition, CONIC - Brazil
Title of study: Intracellular Analysis of DCRA e DCRB Proteins

Thesis Defense Committee

2019 **Brisa C. A. Chagas**
Title of study: Biochemical and structural characterization of recombinant enzymes of the furfural and hydroxymethylfurfural degradation pathway
Advisor: Dr. Ronaldo A. Nagem
Federal University of Minas Gerais, UFMG, Brazil

Academic Productivity

Academic name	Cassago, A	
ORCID ID	0000-0003-1032-4749	
Scopus Author ID	8659227900	(citation count: 792)
Web of Science - Publons ID	AAL-2760-2020	(citation count: 751)
ResearchGate ID	Alexandre-Cassago	(citation count: 938)
Google Scholar	Alexandre-Cassago	(citation count: 1089)
Publication number	23	
h-index	13	

2023 Molecular mechanism of glutaminase activation through filamentation and the role of filaments in mitophagy protection.
Nature Structural & Molecular Biology
DOI: 10.1038/s41594-023-01118-0

2022 Structural dynamics of SARS-CoV-2 nucleocapsid protein induced by RNA binding.
PLOS Computational Biology
DOI: 10.1371/journal.pcbi.1010121

2021 Cryo-EM structure of the mature and infective Mayaro virus at 4.4 Å resolution reveals features of arthritogenic alphaviruses.
Nature Communications
DOI: 10.1038/s41467-021-23400-9

2020 Specimen preparation optimization for size and morphology characterization of nanocellulose by TEM.
Cellulose
DOI: 10.1007/s10570-020-03116-7

2020 Myriapod haemocyanin: the first three-dimensional reconstruction of Scolopendra subspinipes and preliminary structural analysis of S. viridicornis.
Open Biology
DOI: 10.1098/rsob.190258

2019 Characterization of phospholipid vesicles containing lauric acid: physicochemical basis for process and product development.
Heliyon
DOI: 10.1016/j.heliyon.2019.e02648

2019 A revised order of subunits in mammalian septin complexes.
Cytoskeleton
DOI: 10.1002/cm.21569

2018 Cryo-EM structure of the bacteria-killing type IV secretion system core complex from Xanthomonas citri.
Nature Microbiology
DOI: 10.1038/s41564-018-0262-z

2018 Evaluation of siRNA and cationic liposomes complexes as a model for *in vitro* siRNA delivery to cancer cells.
Colloids and Surfaces A: Physicochemical and Engineering Aspects
DOI: 10.1016/j.colsurfa.2018.06.073

2017 Schistosoma mansoni displays an adenine phosphoribosyltransferase preferentially expressed in mature female gonads and vitelaria.
Molecular and Biochemical Parasitology
DOI: 10.1016/j.molbiopara.2017.04.004

2017 Structure and kinetics assays of recombinant Schistosoma mansoni dihydrofolate reductase.
ACTA Tropica
DOI: 10.1016/j.actatropica.2017.03.007

2016 Analysis of two Schistosoma mansoni uridine phosphorylases isoforms suggests the emergence of a protein with a non-canonical function.
Biochimie
DOI: 10.1016/j.biochi.2016.02.007

2015 Structure and mechanism of dimer-monomer transition of a plant poly(A)-binding protein upon RNA interaction: insights into its poly(A) tail assembly.
Journal of Molecular Biology
DOI: 10.1016/j.jmb.2015.05.017

2015 Association between cationic liposomes and low molecular weight hyaluronic acid.
Langmuir
DOI: 10.1021/la5045865

2014 Investigation of *Escherichia coli* Selenocysteine Synthase (SelA) complex formation using cryo-electron microscopy (Cryo-EM).
Microscopy and Microanalysis
DOI: https://doi.org/10.1017/S1431927614008149

2013 Active Glutaminase C Self-assembles into a Supratetrameric Oligomer That Can Be Disrupted by an Allosteric Inhibitor.
Journal of Biological Chemistry
DOI: 10.1074/jbc.M113.501346

2013 Functional Diversification of Cerato-Platanins in Moniliophthora perniciosa as Seen by Differential Expression and Protein Function Specialization.
Molecular Plant-Microbe Interactions
DOI: 10.1094/MPMI-05-13-0148-R

2013 Adenosine kinase from Schistosoma mansoni : structural basis for the differential incorporation of nucleoside analogues.
Acta Crystallographica. Section D, Biological Crystallography
DOI: 10.1107/S0907444912044800

2013 An efficient protocol for the production of tRNA-free recombinant Selecnocysteine Synthase (SELA) from Escherichia coli and its biophysical characterization.
Protein Expression and Purification
DOI: 10.1016/j.pep.2012.12.005

2012 Mitochondrial localization and structure-based phosphate activation mechanism of Glutaminase C with implications for cancer metabolism.
Proceedings of the National Academy of Sciences of the United States of America
DOI: 10.1073/pnas.1112495109

2006 Identification of Leishmania selenoproteins and SECIS element.
Molecular and Biochemical Parasitology
DOI: 10.1016/j.molbiopara.2006.05.002

2005 Gene expression profile of human Down syndrome leukocytes.
Croatian Medical Journal
https://www.ncbi.nlm.nih.gov/pubmed/16100769

2002 Cellophane based mini-prep method for DNA extraction from the filamentous fungus Trichoderma reesei.
BMC Microbiology
DOI: 10.1186/1471-2180-2-14