BIOGRAPHICAL SKETCH

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NAME: Celia Regina Ribeiro da Silva Carlini

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eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Full Professor (visiting)

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Federal University of São Paulo (UNIFESP), São Paulo, Brazil	B.Sc.	12/1978	Biomedical Sciences
Federal University of São Paulo (UNIFESP), São Paulo, Brazil	M.Sc.	02/1981	Molecular Biology – Protein Chemistry
Federal University of São Paulo (UNIFESP), São Paulo, Brazil	Ph.D.	05/1985	Molecular Biology – Protein Chemistry
University of Arizona, Tucson, AZ, USA	Postdoctor	11/1995	Biochemistry – Insect Science

A. Personal Statement

I was born in São Paulo, Brazil, in 1956, the daughter of Elisaldo Luiz de Araujo Carlini (father) and Glaci Ribeiro da Silva (mother). I am a mother of two children, one of which has special needs (Down's syndrome). My parents were scientists active in the field of Pharmacology, and they fostered my curiosity and interest in science since my early childhood. My research interests are centered on studies of structure versus activity relationships and mechanisms of action of toxic proteins, including plant and microbial toxins. During my M.Sc. dissertation, I've isolated and characterized a convulsant protein, which was named canatoxin (CNTX), from jack bean (*Canavalia ensiformis*) seeds. During my Ph.D. thesis, I studied the mechanisms of neurotoxicity and platelet aggregation induced by CNTX and the requirement of lipoxygenase-derived eicosanoids for the toxin's effects. In 1997, I reported that CNTX and derived peptides display insecticidal and fungitoxic effects and may have a role in plant defense. In 2001, my group characterized CNTX as an isoform of the jack bean urease (JBU) and reported that the neurotoxicity and platelet aggregating effect of CNTX and JBU do not require their urea-splitting enzyme activity. These results established the ground for a new field of studies on the enzyme-independent properties of ureases.
The articles below are results from this stage of my scientific career:

• Carlini, C. R..; Guimarães, J. A. Isolation and Characterization of a Toxic Protein from *Canavalia ensiformis* (Jack Bean) Seeds, Distinct From Concanavalin A. Toxicon (Oxford), v.19, p.667 - 675, 1981. PMID 7302956 (cited 102 times, Web-of-Sciences, Dec 27, 2024))

• Carlini, C. R..; Guimarães, J. A.; Ribeiro, J. M. C. Platelet release reaction and aggregation induced by canatoxin, a convulsant protein: evidence for the involvement of the platelet lipoxygenase pathway. British Journal of Pharmacology, v.84, p.551 - 560, 1985. PMID 3919794 (cited 62 times)

• Carlini, C. R..; Oliveira, A. E. A.; Azambuja, P.; Xavier-Filho, J.; Wells, M. A. Biological Effects of Canatoxin in Different Insect Models: Evidence for a Proteolytic Activation of the Toxin by Insect Cathepsinlike Enzymes. Journal of Economic Entomology, v.90, p.340 - 348, 1997. PMID 9145031 (cited 87 times)
• Follmer, C.; Barcellos, G. B. S.; Zingali, R. B.; Machado, O. L. T.; Alves, E. W.; Barja-Fidalgo, C.; Guimarães, J. A.; Carlini, C. R. Canatoxin, a toxic protein of jack beans (*Canavalia ensiformis*), is a variant form of urease (EC 3.5.1.5). Biological effects of urease independent of its ureolytic activity. Biochemical Journal (London. 1984), v.360, p.217 - 224, 2001. PMID 11696010 (cited 79 times).

My research then widened to ureases of other sources, particularly those of pathogenic microorganisms. These studies have shown that ureases potentially contribute to the pathogenesis of diseases caused by urease-positive bacteria and fungi. In the case of the *Helicobacter pylori* urease (HPU), besides neutralizing the acidity in the stomach and enabling bacterial survival, HPU activates platelets, induces angiogenesis, has pro-inflammatory activity, and disrupts the barrier function of endothelial cells, all these effects are mediated by eicosanoid-dependent pathways. Likewise, the *Proteus mirabilis* urease (PMU) displays enzyme-independent pro-inflammatory properties that may potentiate the noxious effects caused by the bacteria during urinary tract infection. More recently, I've initiated studies aiming to elucidate how the neurotoxicity of ureases relates to Alzheimer's (AD) and Parkinson's diseases (PD), pathologies that have been correlated with *H. pylori* infection. I demonstrated that injection of rodents with purified HPU or PMU induces neuroinflammation, affects the behavior and promotes alterations in AD/PD protein markers in the brains of treated animals. HPU is a major component of outer membrane vesicles (OMVs) released by *H. pylori*. These OMVs can cross the blood brain barrier and deliver HPU to the central nervous system.

B. Positions, Scientific Appointments, and Honors

I was an Associate Professor for 17 years (1981-1997) at the Department of Medical Biochemistry, Federal University of Rio de Janeiro (UFRJ). In 1997, I moved with my family to Porto Alegre, RS, in the southern region of Brazil. From 1997 to 2012, I was a Full Professor and Research Group Leader at the Center of Biotechnology, Federal University of Rio Grande do Sul (UFRGS), in Porto Alegre, RS. I retired from UFRGS in 20212, and in 2013 I joined, as Principal Investigator, the Brain Institute of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) in Porto Alegre. In 2022, I moved my laboratory back to UFRGS as an Invited Professor. Since March 2024, I am a Visiting Professor at the Federal University of Medical Sciences of Porto Alegre (UFCSPA). Presently I am a permanent lecturer in graduate programs at UFRGS (Cellular and Molecular Biology; Biochemistry) and UFCSPA (Biosciences), where I teach and supervise graduate students.

Since 2005, I am a Research Productivity fellow, level 1A (highest level), of the Brazilian National Council for Scientific and Technological Development (CNPq).

I was elected a full member of the Brazilian Academy of Sciences, Biomedical Section in 2008, and in 2024 I was elected member of The World Academy of Science (TWAS), Biological Sciences Section.

I have coordinated the Graduate Program in Biological Chemistry at UFRJ (1992-1993) and the Graduate Program in Cellular and Molecular Biology at UFRGS (1998-2002; 2005-2009), both ranked among the best in the country. So far, I concluded the supervision of 32 M.Sc. dissertations, 36 Ph.D. thesis, and 20 postdoctoral trainees. Today, my former students hold tenure positions at other high-education Brazilian institutions; some work in biotechnology companies or are undertaking postdoctoral training in Brazil or abroad.

My expertise has been recognized with grants from Brazilian agencies and three from the International Foundation for Science (Sweden). I coordinated multi-institutional Brazilian grants such as PADCT, PRONEX and "Edital Toxinologia" (CAPES), and a subproject of the National Institute of Brain Diseases, Excitotoxity and Neuroprotection (INCT). The continuous support received from the Brazilian National Council for Scientific and Technological Research, and the state agency Foundation for Research Support of the Rio Grande do Sul (FAPERGS), are testament of the significant contributions I've made to the field.

For several years, I have participated in Committees of the Coordination for the Improvement of Higher Education Personnel (CAPES) from the Ministry of Education, Brazil. CAPES is the government organ that evaluates, accredits and finances graduate programs in the whole country. I was elected twice to the Advisory Committee in Biochemistry, Biophysics, Pharmacology, Physiology, and Neurosciences, Brazilian National Council for Scientific and Technological Development (CNPq) (2007-2009; 2022-2026). I was a member of the Editorial Board of Toxicon, the official journal of

the International Society on Toxinology, for about 20 years, and acted as Sectional Editor (Biochemistry) of the Brazilian Journal of Medical and Biological Research. Presently I am an Executive Editor of Current Neuropharmacology (2024-2027). I have refereed for more than 60 international journals and reviewed grant applications for Brazilian (CNPq, CAPES, FINEP, FAPESP, FAPERJ, FIOCRUZ, EMBRAPA, among others) as well as international agencies (A*Star, Singapura; Narodowe Centrum Nauki, Poland; Flandres Research Foundation; Agence Nationale de la Recherche, France; US Department of Agriculture).

C. Contributions to Science

I have published 12 book chapters and 149 papers in international journals with a peer-review policy. I edited two books and acted as guest editor for special volumes of journals. The 169 documents authored by me and indexed in Web-of-Sciences received 4010 citations (Dec 27, 2024), with a H factor=34. In the Scopus database, the 148 documents authored by me received 4429 citations, with a H factor=37.
On the Web-of-Sciences database, a search using the query “urease” in the “title” retrieved 5,895 documents, 75 of which are authored by myself (Celia R Carlini). This places me in the fourth position among authors with the most papers fulfilling this search criteria. The same search conducted on the Scopus database also puts me in the fourth position among authors with the most papers with “urease” in the title. These bibliometric data indicate that my contribution to the field of “urease” is relevant. It is particularly outstanding as many of these articles are authored solely by Brazilians, and most research was conducted entirely in Brazilian universities. The main contributions, organized into five topics, and representative articles of the key findings of my research on ureases are indicated below. I am the first, last, or correspondent author in all these papers. Students supervised by me co-authored most of these articles.

1. Ureases are multifunctional proteins endowed with non-enzymatic biological properties

In 1981, I isolated from jack bean (Canavalia ensiformis) seeds a neurotoxic protein canatoxin (CNTX) that induces seizures in rodents given by ip or ev route. In 1985, I discovered that CNTX promotes aggregation of blood platelets dependent on exocytosis and mediated by the platelet 12-lipoxygenase. From 1981 up to 2000, my team studied the mechanism of neurotoxicity, exocytosis-inducing and proinflammatory action of CNTX, and the requirement of lipoxygenase-derived eicosanoids for most of the toxin’s effects. In 2001, we characterized CNTX as an isoform of the jack bean urease (JBU). This discovery widened the research field to ureases from different sources and with different quaternary structures: single-chained ureases from plants (soybean, cotton) and fungi (*Cryptococcus gattii*), and bacterial ureases with two (*Helicobacter pylori*) or three (*Bacillus pasteurii, Proteus mirabilis*) subunits. Urease-induced biological effects independent of urea-splitting enzyme activity were demonstrated for all of them. These results established the ground for a new field of studies on the enzyme-independent properties of ureases.

- Grahl, Matheus V. C.; Uberti, Augusto F.; Broll, Valquiria; Bacaicoa-Caruso, Paula; Meirelles, Evelin F.; Carlini, Celia R *Proteus mirabilis* Urease: Unsuspected Non-Enzymatic Properties Relevant toPathogenicity. International Journal of Molecular Sciences, v.22, p.7205 - 7222, 2021. PMID 34281258
- Olivera-Severo, Deiber; Uberti, Augusto F.; Marques, Miguel S.; Pinto, Marta T.; Gomez-Lazaro, Maria; Figueiredo, Céu; Leite, Marina; Carlini, Celia R. A New Role for *Helicobacter pylori* Urease: Contributions to Angiogenesis. Frontiers in Microbiology, v.8, p.1883, 2017. PMID 29021786
- Olivera-Severo, Deiber; Wassermann, German E.; Carlini, Celia R. *Bacillus pasteurii* urease shares with plant ureases the ability to induce aggregation of blood platelets. Archives of Biochemistry and Biophysics (Print), v.452, p.149 - 155, 2006. PMID 16839515
- Piovesan, A. R.; Martinelli, A. H. S.; Ligabue-Braun, R.; Schwartz, J-L.; Carlini, Celia R. *Canavalia ensiformis* urease, Jaburetox and derived peptides form ion channels in planar lipid bilayers. Archives of Biochemistry and Biophysics, v.547, p.6 - 17, 2014. PMID 24583269

2. Structure versus Activity Relationships in Ureases and Derived Peptides

I have addressed two aspects of structure versus activity of ureases. The first aspect deals with distinguishing the three isoforms of jack bean ureases: CNTX, JBU, and JBURE-II. The second focuses on mapping the biologically active domains of ureases or their derived peptides. These studies were carried out with (1) CNTX- and JBU-enzymatically derived peptides; (2) the isolated subunits of the di-chained *Helicobacter pylor*i urease; (3) the isolated subunits of the tri-chained *Proteus mirabilis* urease; and (4) mutants of jaburetox, a JBU-derived recombinant peptide with antifungal and insecticidal properties.

• Moro, Carlo F.; Nogueira, Fabio C. S.; Almeida, Carlos G.; Real-Guerra, Rafael; Dalberto, Pedro F.; Bizarro, Cristiano V.; Ligabue-Braun, Rodrigo; Carlini, Celia R. One enzyme, many faces: urease is also canatoxin. Journal of Biomolecular Structure & Dynamics, p.1 - 12, 2022. PMID 36546698
• Broll, Valquiria; Perin, Ana Paula A.; Lopes, Fernanda C.; Martinelli, Anne H. S.; Moyetta, Natalia R.; Fruttero, Leonardo L.; Grahl, Matheus V. C.; Uberti, Augusto F.; Demartini Diogo R.; Ligabue-Braun, Rodrigo; Carlini, Celia R. Non-Enzymatic Properties of Proteus mirabilis Urease Subunits. ProcessBiochemistry, v.110, p.263 - 274, 2021. https://doi.org/10.1016/j.procbio.2021.08.023
• Martinelli, Anne H. S.; Kappaun, Karine; Ligabue-Braun, Rodrigo; Defferrari, Marina S.; Piovesan, Angela R.; Stanisçuaski, Fernanda; Demartini Diogo R.; Verli, Hugo; Dal Belo, Chariston A.; Almeida, Carlos G.; Follmer, Cristian; Carlini, Celia R.; Pasquali, Giancarlo. Structure-function studies on Jaburetox, a recombinant insecticidal and antifungal peptide derived from jack bean (*Canavalia ensiformis*) urease. Biochimica et Biophysica Acta-General Subjects, v.1840, p.935 - 944, 2014. PMID 24239686
• Mulinari F., Becker-Ritt Arlete, Demartini, Diogo R., Ligabue-Braun, Rodrigo, Stanisçuaski, Fernanda, Verli, Hugo, Fragoso, Rodrigo R, Schroeder, Evelyn K., Carlini, Celia R., Grossi-de-Sá, Maria Fatima. Characterization of JBURE-IIb isoform of *Canavalia ensiformis* (L.) DC urease. Biochimica et Biophysica Acta – Protein and Proteomics, v.1814, p.1758-68. PMID 21893219

3. Ureases and derived peptides have insecticidal and fungitoxic actions unrelated to enzymatic activity, and their potential biotechnological use in agriculture.

Studies on the insecticidal effect of CNTX indicated that the protein requires a proteolytic step to release an internal peptide with entomotoxic activity. Two recombinant versions of this peptide (jaburetox and soyuretox) displayed insecticidal and antifungal activities, revealing potential as biopesticides. The recombinant peptides were structurally characterized as intrinsically disordered and shown to interact with lipid bilayers, thereby affecting their biophysical properties.

• Kappaun, Karine; Martinelli, Anne H. S.; Broll, Valquiria; Zambelli, Barbara; Lopes, Fernanda C.; Ligabue-Braun, Rodrigo; Fruttero, Leonardo L.; Moyetta, Natalia R.; Bonan, Carla D.; Carlini, Celia R.; Ciurli, Stefano. Soyuretox, an Intrinsically Disordered Polypeptide Derived from Soybean (*Glycine max*) Ubiquitous Urease with Potential Use as a Biopesticide. International Journal of Molecular Sciences, v.20, p.e5401, 2019. PMID 31671552
• Lopes, Fernanda C.; Dobrovolska, Olena; Real-Guerra, Rafael; Broll, Valquiria; Zambelli, Barbara; Musiani, Francesco; Uversky, Vladimir N.; Carlini, Celia R.; Ciurli, Stefano. Pliable natural biocide: Jaburetox is an intrinsically disordered insecticidal and fungicidal polypeptide derived from jack bean urease. FEBS Journal, v.282, p.1043-64, 2015. PMID 25605001
• Wiebke-Strohm, Beatriz; Pasquali, Giancarlo; Pinheiro-Margis, Marcia M. A. N.; Bencke, M.; Bucker-Neto, L.; Becker-Ritt, Arlete B.; Martinelli, Anne H. S.; Rechenmacher, Cialiana; Polacco, Joseph C.; Stolf, R.; Marcelino, F. C.; Homrich, M. S.; Del Ponte, E. M.; Carlini, Celia R.; De Carvalho, M. C. C. G.; Bodanese-Zanettini, Maria H. Ubiquitous urease affects soybean susceptibility to fungi. Plant Molecular Biology, v.79, p.75 - 87, 2012. PMID 22382992
• Becker-Ritt, Arlete B.; Martinelli, Anne H. S.; Mitidieri, S.; Feder, Vanessa; Wassermann, German E.; Santi, Lucélia; Vainstein, Marilene H.; Oliveira, Jose T. A.; Fiuza, Lidia M.; Pasquali, Giancarlo; Carlini, Celia R. Antifungal Activity of Plant and Bacterial Ureases. Toxicon (Oxford), v.50, p.971 -983, 2007. PMID 17825863.

4. Ureases promote exocytosis and inflammation mediated by lipoxygenase-derived eicosanoids

The exocytosis-inducing effect on blood platelets, mediated by lipoxygenase-derived eicosanoids, was first described for CNTX and then extended to soybean, *B. pasteurii, H. pylori*, and *P. mirabilis* ureases. Induction of exocytosis by CNTX was later described in pancreatic β-cells, macrophages, mastocytes, and in synaptosomes for CNTX and JBU. The pro-inflammatory eicosanoid-mediated effect in the paw edema model was described for CNTX and *H. pylori* urease. The production of reactive oxygen species and of the pro-inflammatory cytokines TNF-α and IL-1β was reported in several cell types for *H. pylori* and for *P. mirabilis* ureases. The data highlight the so far overlooked direct pro-inflammatory (non-enzymatic) effect of microbial ureases that might contribute to diseases caused by urease-positive pathogens.

• Souza, Mariele; Moraes, João A.; Da Silva, Vany N.; Helal-Neto, Edward; Uberti, Augusto F.; Scopel-Guerra, Adriele; Olivera-Severo, Deiber; Carlini, Celia R.; Barja-Fidalgo, Christina. *Helicobacter pylori* urease induces pro-inflammatory effects and differentiation of human endothelial cells: Cellular and molecular mechanism. Helicobacter, v.24, p.e12573, 2019. PMID 30907046

• Scopel-Guerra, Adriele; Olivera-Severo, Deiber; Staniscuaski, Fernanda; Uberti, Augusto F.; Callai-Silva, Natália; Jaeger, Natalia; Porto, Barbara N.; Carlini, Celia R. The Impact of *Helicobacter pylori* upon Platelets and Consequent Contributions to Inflammation. Frontiers in Microbiology, v.8, p.1 - 13, 2017. PMID 29312166
• Uberti, Augusto F.; Olivera-Severo, Deiber; Wassermann, German E.; Scopel-Guerra, Adriele; Moraes, João A.; Barcellos-de-Souza, P.; Barja-Fidalgo, Christina; Carlini, Celia R. Pro-inflammatory properties and neutrophil activation by *Helicobacter pylori* urease. Toxicon, v.69, p.240 - 249, 2013. PMID 23466444
• Wassermann, German E.; Olivera-Severo, Deiber; Uberti, Augusto F.; Carlini, Celia R. *Helicobacter pylori* urease activates blood platelets through a lipoxygenase-mediated pathway. Journal of Cellular and Molecular Medicine v.14, p.2025 - 2034, 2010. PMID 19754669

5. Ureases and derived peptides are neurotoxic to rodents and to insects

Initial studies on the mechanism of neurotoxic action of CNTX in rodents revealed that the protein causes CNS-generated seizures, hypoxia, and hypoglycemia. Later, the neurotoxic effect of CNTX and JBU was further characterized, showing that the proteins induce L-glutamate release, affect the electroencephalographic pattern, and inhibit long-term potentiation. MicroPET studies indicated that JBU promotes activation of the rat brain glucose metabolism in areas consistent with its seizure-inducing activity. The insecticidal effect of JBU and jaburetox also has a neurotoxic component, with insect behavior disturbance, and neuromuscular junction blockade. More recently, my group began studies on the neuroinflammation and neurotoxicity promoted by *H. pylori* and *P.mirabilis* ureases and the possible contribution of these ureases to Alzheimer's and Parkinson's diseases.

• Grahl, Matheus V. C.; Andrade, Brenda S.; Perin, Ana P. A.; Neves, Gilda A.; Duarte, Laura S.; Uberti, Augusto F.; Hohl, Kelvin S.; Follmer, Cristian; Carlini, Celia R. Could the Urease of the Gut Bacterium *Proteus mirabilis* Play a Role in the Altered Gut-Brain Talk Associated with Parkinson's Disease?. Microorganisms, v.11, p.2042 - , 2023. PMID 37630602
• Uberti, Augusto F.; Callai-Silva, Natalia; Grahl, Matheus V. C.; Piovesan, Angela R.; Nachtigall, Eduarda G.; Furini, Cristiane R. G.; Carlini, Celia R. *Helicobacter pylori* Urease: Potential Contributions to Alzheimer's Disease. International Journal of Molecular Sciences, v.23, p.3091, 2022. PMID 35328512
• Almeida, Carlos G. M.; Higuchi, Kiyo C.; Piovesan, Angela R.; Moro, Carlo F.; Venturin, Gianina T.; Greggio, Samuel; Costa-Ferro, Zaquer S.; Salamoni, Simone D.; Peigneur, Steve; Tytgat, Jan; De Lima, Maria E.; Silva, Carolina N.; Vinadé, Lúcia; Rowan, Edward G.; Dacosta, Jaderson C.; Belo, Chárist on A. Dal; Carlini, Celia R.. Neurotoxic and convulsant effects induced by Jack Bean Ureases on the Mammalian Nervous System. Toxicology, v.454, p.152737, 2021. PMID 33631299
• Carrazoni, Thiago; Nguyen, C.; Maciel, L. F.; Delgado-Canedo, A.; Stewart, B. A.; Lange, Angela B.; Dal Belo, Chariston A.; Carlini, Celia R.; Orchard, Ian. Jack bean urease modulates neurotransmitter release at insect neuromuscular junctions. Pesticide Biochemistry and Physiology, v.146, p.63 - 70, 2018. PMID 29626993