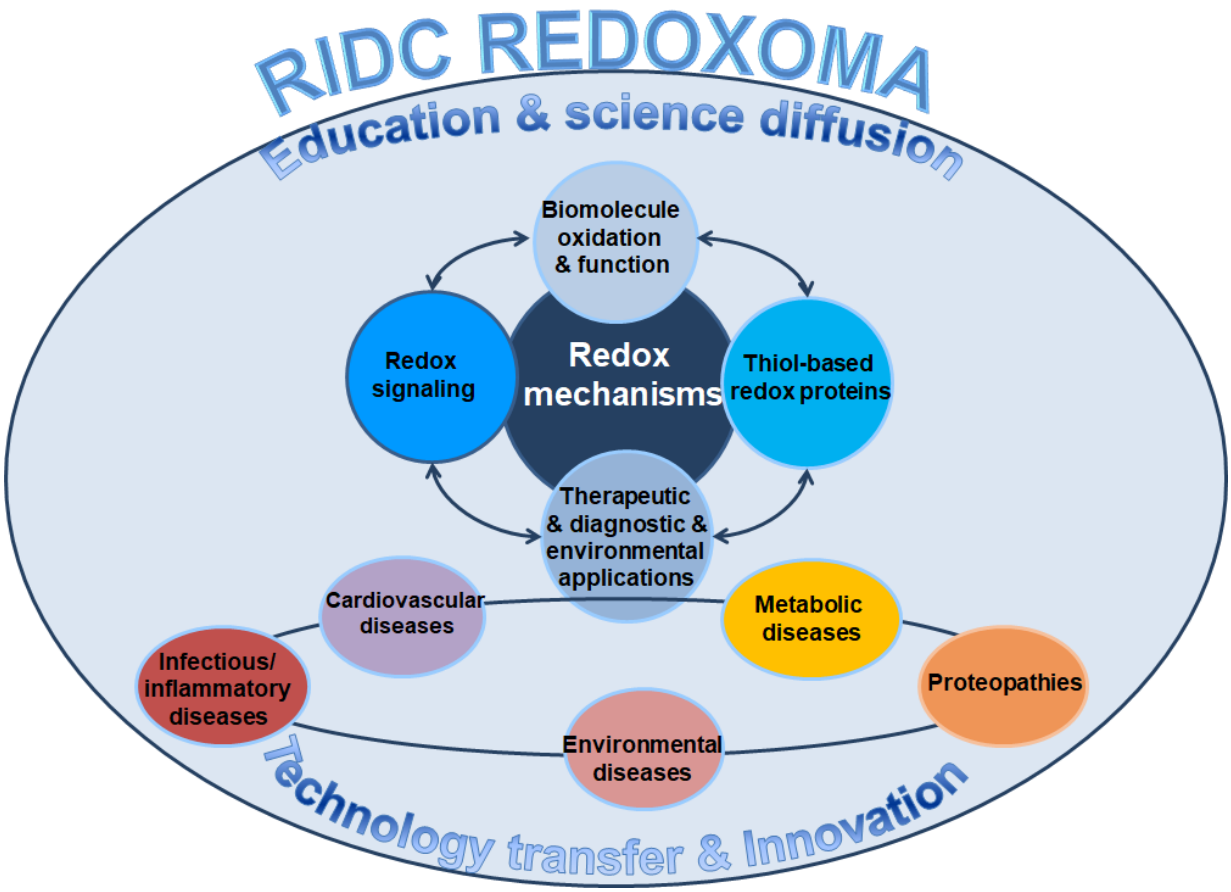
Scientific, Societal and Economic Impact

Center for Research on Redox Processes in Biomedicine (Redoxoma)

http://redoxoma.iq.usp.br/

FAPESP # 2013/07937-8



August, 2013–July, 2018

Scientific, Societal and Economic Impact of RIDC Redoxoma (2013-2018)

RIDC Redoxoma has the mission to carry out scientific research on Redox Biology that impacts not only human knowledge but also the Brazilian society and economy, a premise of the RIDC Program at FAPESP. Since its creation in 2013, RIDC Redoxoma worked to fulfill its mission by increasingly integrating its members and corresponding research, dissemination and innovation activities. Such integration is only possible in a structure (Center) composed by organized researchers with different specialties and stably supported for a considerable period of time. As consequence, the research performed by RIDC Redoxoma already impacted knowledge, society and economy.

Before presenting these impacts, it is important to note that quantitative measurements of scientific, societal and economic impact are quite complex, as extensively argued in the literature. Even more quantifiable parameters used to measure impact, that is, the impact on knowledge, are not free of controversies. Despite the existence of datasets that enable the calculation of bibliometric values such as the *h* index or journal impact factor, the relevance of these parameters to measure scientific impact remains disputed in the scientific community. The situation is even more complex for societal and economic impact. The social impact of research receives variable meanings and definitions and tools to predict and quantify it remain a major challenge (Bornmann, EMBO Rep. 2012; 13:673). Quantification of the economic impact of any activity/product is a branch of Economy, requiring specialists to evaluate it. RIDC`s researchers have a medical, biological or chemical background and are unable to adequately quantify the economic impact of the performed research. Additionally, impact analysis is more revealing in the range of decades than in the range of a few years. Despite all of these difficulties, we can demonstrate that the scientific, societal and economic impact of RIDC Redoxoma´s research was considerable in less than 6 years.

Scientific Impact

The interaction among the Center´s researchers matured and the Center already made relevant discoveries on redox mechanisms, providing new diagnostic and therapeutic targets for translational applications. Here we list novel discoveries most likely to generate further scientific, societal and/or economic impact.

Photosensitized oxidations in health - Photosensitized oxidations, which are reactions promoted by the interaction of light with a photosensitizer (PS) molecule, have well-known detrimental biological effects, *e.g.* skin aging and cancer. Remarkably, medical technologies, such as photodynamic therapy, have also been ingeniously developed for exploring these reactions to trigger oxidation of biomolecules and, consequently, eliminate cancer cells or pathogens. Nevertheless, the detailed molecular steps leading to biological injury remain largely uncharacterized. In a recent publication we demonstrated that for a PS to fully compromise membrane function, it needs to be sacrificed through contact-dependent reactions, forming lipid-truncated aldehydes, which are the active agents causing membrane leakage (Bacellar et al, J Am Chem Soc 2018; DOI: 10.1021/jacs.8b05014). Therefore, activation/suppression of PS regeneration could be explored as an effective tool to maximize or counter the effects of photosensitized oxidations. This concept is allowing new directions in the development of improved photosensitizers (Albani et al, J Am Chem Soc 2014; 136:17095) and in the development of improved protocols for the treatment of the diabetic foot (Tardivo et al, PLOS One 2015; 10: e0135707), as well as in the avoidance of photodamage in sun screens that protects against visible light (Tonolli et al, J Invest Dermatol 2017; 137:2447). These results were publicly announced in two of the most prestigious international meetings in Photochemistry and Photobiology as invited speeches (2018 Biennial Meeting American Society for Photobiology and 2018 Inter-American Photochemical Society Meeting).

Potential target for antibiotic development - Oxidants play central roles in cell signaling during host-pathogen interactions. Among them, arachidonic acid hydroperoxides are mediators of inflammatory processes in mammals, whereas linoleic acid hydroperoxides play equivalent roles in plants. Therefore, hydroperoxide levels are strictly controlled by both hosts and pathogens. RIDC Redoxoma is heading the biochemical/structural characterization of bacterial Ohr (Organic Hydroperoxide Resistance Protein), which is a unique Cys-based peroxidase with very distinct features from analogous proteins in host organisms. Recently, we showed that Ohr reduces fatty acid hydroperoxides and peroxynitrite with extremely high rate constants (Alegria et al, Proc Natl Acad Sci 2017; 114:E132). Furthermore, bacterial mutants devoid of the Ohr gene display high sensitivity to these oxidants, whereas mutant strains deficient for other peroxidases were equally sensitive to fatty acid hydroperoxides, compared to wild type cells. Therefore, Ohr plays central roles in bacterial response to two hydroperoxides that are at the host–pathogen interface. Ohr enzymes were previously thought to be restricted to prokaryotes, but we found Ohr orthologues also in eukaryotic

microorganisms (some pathogenic), many of them located in mitochondria (Meireles et al, Redox Biol 2017; 12:600). OhrR is a transcriptional regulator that represses Ohr expression. We recently showed OhrR also represses diguanylate cyclase expression in *Chromobacterium violaceum*. Furthermore, deletion of OhrR attenuated the virulence of *C. violaceum* in mice, decreasing the bacterial burden in the liver (Previato-Mello et al, Infec Immun 2017, 85: e00017-17). Since the Ohr-OhrR system is absent in both mammals and plants, it represents an attractive target for drug development. As consequence of this work, Luis Netto was invited as a Discussion leader of the Gordon Research Conference of Thiol-Based Redox Regulation and Signaling, 2018.

Diets and mitochondrial calcium homeostasis - In 2017, we discovered that preventing obesity through caloric restriction in rats and mice increases brain mitochondrial calcium uptake capacity and velocity (Amigo et al., Aging Cell 2017; 16:73). Soon after, the same results were found in liver mitochondria (Menezes-Filho et al., Free Radic Biol Med 2017; 110:219). In both organs, the increased mitochondrial calcium buffering capacity is associated with tissue protection. This finding represents a novel and important advance because both caloric intake and calcium homeostasis are central metabolic regulators. This is the first evidence, to our knowledge, that a dietary intervention can change calcium transport in mitochondria, the central hubs of metabolism. Our finding thus uncovers a new and important mechanism in which dietary interventions affect age-related disease, and is in line with the central aim of RIDC Redoxoma to identify new mechanisms in Redox Biology. We now plan to further investigate the biological consequences of diet-induced changes in calcium homeostasis, both in physiology and pathology of different organs. The importance of this finding is illustrated by the fact that the original article from 2017 already has 10 citations (excluding self-citations). The results were presented as the opening talk in the 2018 Gordon Conference on NADHP Oxidases, a plenary PABMB (Pan-American Biochemistry and Molecular Biology) Lecture during the 2018 Federation of the European Biochemical Societies (FEBS) Conference, as well as in the 2018 Brazilian Academy of Sciences (ABC) and Brazilian Society for the Progression of Science (SBPC) meetings.

Protein disulfide isomerase-A1 as a novel mediator of vascular remodeling - Research from RIDC Redoxoma identified a novel redox mechanism underlying vascular remodeling, a fundamental but poorly understood disease process controlling arterial caliber and atherosclerosis progression. We had previouly described novel roles of protein disulfide isomerase-A1 (PDIA1), a redox chaperone mainly from the endoplasmic reticulum, in vascular smooth muscle cell (VSMC) signaling and NADPH oxidase-related migration (reviewed in Soares-Moretti & Laurindo, Arch Biochem Biophys 2017; 617:106). Given the slow PDIA1 reaction rates with peroxides (Peixoto A et al, JBC 2018; 293:1450), we proposed that PDIA1 acts as a molecular redox adaptor rather than mass peroxide sensor. On such basis, we postulated that PDIA1 regulates vascular remodeling. Using data from human specimens, animal and in vitro models, we discovered that extracellular PDIA1 supports expansive vessel remodeling through effects on cytoskeletal and extracellular matrix regulation and identified β1 integrin as PDIA1 target (Tanaka L et al, Hypertension 2016; 67:613). Possible mechanisms of these effects involve a novel interaction between PDIA1 and RhoGTPases (known cytoskeletal regulators) (Pescatore L et al, J Biol Chem 2012), and we showed that a cluster of adjacent genes coding for PDI and RhoGDI (a crucial RhoGTPase regulator) is conserved for >800-million years (Moretti A et al, Sci Rep 2017; 7:17262). Routes of PDIA1 externalization in vascular cells, highly debated in the literature, were shown to be unconventional (Araujo T et al, Free Radic Biol Med 2017; 103:199). Additional investigation under review disclosed novel roles of PDIA1 as master organizer of VSMC phenotype switch and of mechano-dependent cytoskeletal organization, both pathological remodeling mechanisms. Such data led to the proposal of a new PDI redox/ mechanosignaling model of vascular remodeling (Tanaka L & Laurindo F, Free Radic Biol Med 2017; 109:11). This work's impact is reflected by an Editorial Comment to our article (Hypertension, 2016), invited reviews, meeting presentations (2 Gordon Conferences, EMBO Redox Conference), chairing of a Gordon Research Conference (2016, USA) and recent election to organize a FASEB conference in 2023, USA.

Societal Impact

RIDC Redoxoma research contributes mainly to social issues relevant to population education, health and well-being. We describe here the contributions in which the social impact will be lasting.

Dissemination and education - The science education and dissemination branch of RIDC Redoxoma articulates scientific research, educational research, teaching across all levels and outreach activities aiming to increase the scientific culture of the Brazilian Society. The following actions and respective products or

audience are highlighted: training of the next generation of researchers (48 PhD, 26 Master and 26 PD degrees completed); continuing education courses for in-service high school science teachers (a total of 170 in-service teachers enrolled in 5 of such courses); publishing work in science education forums (close to 40 works); participating in videos directed to the general public (12 videos shared in the You Tube that reached 18,552 visualizations up to July 30, 2018); Free and Radical web portal (1,600 registered users and 2,300 web sessions); visually appealing experiments in science fairs (more than 5,000 visitors); publicity campaign in the São Paulo subway system (red and green line passengers); two commercial books for the general public on aspects of Redox Biology; seminars for the general public in forums such as Pint of Science. With this range of education and dissemination activities reaching several thousands of participants, RIDC Redoxoma is certainly contributing to increase Brazilian Society awareness for science and technology.

Health and well-being- Research on caloric restriction has led to important insights into mechanisms and limitations - and even potential harms - of specific diets (Kowaltowski et al, several articles) and the related information has been transferred to the general public through the web, a book, television, seminars and other media. Dissemination of such knowledge is of prime importance considering the epidemics of obesity, insulin resistance and associated diseases worldwide and in Brazil.

Additional research has provided advances into the treatment of the diabetic foot, a process responsible for amputations in a target population potentially reaching approximately 3 million individuals in Brazil. These collaborative studies have so far covered > 300 patients, in many cases avoiding amputations and their significant consequences. Protocols developed by our group use photodynamic therapy (Tardivo J et al, Photodiagn Photodynamic Ther 2014; 11:342) and are now programed to undergo a multicenter Phase 3 efficacy randomized trial.

Cardiovascular diseases account for the majority of deaths in Brazil and worldwide. Research from Redoxoma has uncovered roles for protein disulfide isomerase (PDIA1) in vascular remodeling. We recently validated a novel ELISA assay to assess PDIA1 levels in human plasma and showed their correlation with plasma proteomic signature and endothelial phenotype (Oliveira P. et al, in preparation). A new preclinical project addressing effects of PDIA1 inhibition in aortic aneurysms is ongoing. These are further examples of mechanistic studies from Redoxoma potentially finding their way into diagnostic and therapeutic applications. Similarly, studies from RIDC Redoxoma showed the potential effect of beta-alanine supplementation in skeletal muscle performance (Carvalho et al, Redox Biol 2018; 18:222). This supplementation enhances the levels of carnosine, which supports detoxification of the aldehyde acrolein in the skeletal muscle. These results are relevant to the performance of competitive athletes, and can also benefit the improvement of muscle performance in aging and diseases accompanied by sarcopenia.

Air pollution is a major environmental risk for human health. Our research documented unequivocally the formation of acetaldehyde-DNA adducts in vivo in mice exposed to particulate matter from the city of São Paulo (Sanchez A et al, Chem Res Toxicol 2018; 31:332). This is an important warning to environmental agencies regarding public health policies, since such adducts are relevant mechanisms in mutagenesis and carcinogenesis.

Increase in the yield of Brazilian crops is important for population food security but also for ensuring the prominent role of agriculture in the Brazilian trade balance with the outside world. RIDC Redoxoma research showed that mixtures of microbes or single microbes enhance nutrient use efficiency by plants (Béltran-Garcia et al Sci Rep 2014; 4:6938; Silva et al, J Plant Growth Regul 2018; 8, 1). These findings are being further explored to decrease chemical fertilizer expenditure.

In addition to the above-discussed social implications of scientific advances from our RIDC Redoxoma, socio-economic impact has also been derived from the Redoxoma Analysis Platform, which serves not only Redoxoma members, but also members of the community at-large. This has been the case of our Lipidomic facility coordinated by Sayuri Miyamoto, which has analyzed more than a thousand samples (since 2016) from research laboratories, industries and hospitals and fostered new advances in precision medicine, e.g., in patients with cancer.

Economic Impact

As pointed out above, we can only grossly estimate the economic impact generated by RIDC Redoxoma research. To this end, we focused on products to which monetary values can be attributed through the market and/or other sources and made a rough estimation of revenues/economy. The results show already a

considerable economic impact of RIDC Redoxoma research (at least **R$ 81.4 million**) and such value can greatly increase over the years.

Training qualified professionals - A considerable number of researchers egressed from the RIDC Redoxoma are in the Brazilian job market (total of 48; 10 Msc, 25 PhD and 13 PDs). Assuming an average salary of R$ 5,000.00/month for each of the professionals (13 months/1 year) and a multiplier 1.73* for direct and induced effects, this adds-up to **R$ 5.4 Mi/year**. The real value should be higher because some of the professionals reached the market some years ago.

(*) The value of 1.73 is the average of multipliers to account for plausible indirect and induced effects, which are suggested by the British Arts Festival Association (1.99), the Treasury (1.7) and Wyndham Report for the Society of London Theatre (1.5).

Diabetic foot treatment – To date, 300 amputations have been avoided due to this project. Direct costs in amputation amount to R$ 4,000.00 per patient and for 300 patients, **R$ 1.2 million**. Adding the indirect benefits of having an active, working, family member (300 patients x 120 months* x R$ 2,000.00/month x patient), this adds-up to **R$ 73.2 million**. If the Phase III trial that we will conduct proves the efficacy of the therapy and it gets approved by the regulatory agencies, the treatment could provide a huge economy (c.a. 1 million potential target patients** corresponding to c.a. **R$ 244 Bi**) to the public health system that can direct it to other urgent needs of the Brazilian population.

**Average work time of a diabetic patient - 10 years (120 months).*
***The total number of Brazilian diabetic patients estimated to require amputation is c.a. 3 million. It is thus reasonable to assume 1million potential target patients for the public health system.*

Reactor based on advanced oxidation processes – This reactor was designed for cleaning contaminated water, constructed and patented **(**BR10 2016 014409-4).The reactor was constructed and tested in Lubrasil Lubricants Ltda, Piracicaba, SP; it reduced the cost of $1m^3$ of water from R$ 215,00 to R$ 23,29 due to water recycling (source: Lubrasil). This represented an economy of around **R$ 700,000.00/year**. Since the reactor is being used since 2014, the total economy amounts to **R$ 2.8 million**. Therefore, the licensing and use of this technology to build similar reactors can generate considerable revenues to Lubrasil, to USP and to other Companies.

Sun screen technology. Several companies are working with CEPID Redoxoma to develop improved sun protection agents, especially those that protect against visible light (BR102016024262-2). A global market of US$ 24.9 billion by 2024 is predicted for these solar protectors (US$ 2 billion in Brazil). Assuming that the agents we are developing will play a major role in the improved sun protection agents they will represent *c.a.* **R$ 100 million** to the cosmetic market.

Consulting and other research agreements
A closer relationship with Companies in a possible second phase of the RIDC Redoxoma is likely to generate considerable economic revenues.