

# Curriculum Vitae

ENGLISH AND GREEK

## **Paschalis-Thomas Doulias, Ph.D.**

### Curriculum Vitae

January 2024

**Position:** Tenured Assistant Professor of Biochemistry

**Work Address:**

Room X3-106d, Laboratory of Biochemistry  
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Additional affiliation: Institute of Biosciences, University Research Center of Ioannina (U.R.C.I.),  
Ioannina

**Education**

**1993-1998:** BS Chemistry, Department of Chemistry, University of Ioannina, Greece

**1999-2005:** PhD, Laboratory of Biological Chemistry, Medical School, University of Ioannina,  
Greece.

**Military Service**

**2004-2005:** One year service in Greek Army

**Postgraduate Training and Fellowship Appointments**

**2011-2014:** Research Associate, the Division of Neonatology at The Children's Hospital of  
Philadelphia Research Institute (CHOP-IR), Philadelphia, PA, USA. Mentor: Harry Ischiropoulos, PhD

**2007-2011:** Postdoctoral fellow, at CHOP-IR, Philadelphia, PA, USA. Mentor: Harry  
Ischiropoulos, PhD

**2005-2006:** Postdoctoral fellow Laboratory of Anatomy-Histology-Embryology, Medical School,  
University of Ioannina, Greece, Mentor: Kanavaros Panayiotis, MD, PhD

**Academic Appointments**

**2019-2023:** Assistant Professor of Biochemistry in tenure track, Department of Chemistry,  
University of Ioannina, Greece

**2017-2019:** Assistant Professor of Pediatrics, Perelman School of Medicine, University of  
Pennsylvania, Philadelphia, PA

**2014-2017:** Instructor of Pediatrics, Perelman School of Medicine, University of Pennsylvania,  
Philadelphia, PA, USA

**Teaching and Training Experience**

**(A)Teaching in Greece (2019- current)**

Undergraduate studies program, Department of Chemistry

*(a) Obligatory Courses*

- Biochemistry I
- Biochemistry II
- Laboratory of Biochemistry
- Undergraduate Thesis (Supervisor of 13 Theses, 12 have been completed, 1 is pending)

*(b) Optional courses*

- Biochemistry III: The regulation of metabolism. From Chemistry to Pathophysiology (course coordinator)
- Biological Membranes and Signal transduction (Course coordinator)
- Advanced laboratory of Biochemistry

Post graduate studies program, Department of Chemistry

- Advanced Biochemistry, Modern Topics in Biochemistry (course coordinator)
- Biological Membranes: Structure, organization, function and cell signaling.
- Laboratory of Biochemistry I & II

Inter-institutional Interdepartmental Program of Postgraduate Studies (I.I.P.S) Molecular and Cellular Biology and Biotechnology, University of Ioannina, Greece.

- Topics in Molecular and Cellular Biology
- Functional Analysis of Genes: from the Design to the Living System (Course vice-coordinator)

Teaching and Training activities as PhD candidate, Laboratory of Biological Chemistry, Medical School, UoI

1999-2003: Laboratory Assistant for “General Chemistry”, “Biochemistry I” and “Biochemistry II”

2002: Supervisor of research activities, undergraduate thesis of a third-year student of Elective Study Programs in Biochemistry

2000-2001: Laboratory Assistant, obligatory course “Biochemistry I”, Elective Study Programs in Biochemistry

2000-2001: Laboratory Assistant, course “Biochemistry of Free Radicals”

**(B) Teaching in USA (2007-2019)**

**2009-2015:** Supervision of research activities of Karthik Raju, PhD candidate, Neuroscience graduate group, University of Pennsylvania. Thesis title «**Regulation of the Glutamate/Glutamine cycle by nitric oxide in the central nervous system**»

**2007-2011:** Supervision of research activities of Jennifer L Greene, PhD candidate Biochemistry and Molecular Biophysics graduate group, University of Pennsylvania. Thesis title «**The structural proteomics of S-Nitrosylation: From global identification to elucidating protein function through structural bioinformatics.**»

**2011-2014:** Supervision of rotation students: Madhu Yennawar, PhD candidate, Pharmacology graduate group, University of Pennsylvania (2014), Maria Fasolino, PhD candidate Neuroscience graduate group, University of Pennsylvania (2012), Wade Mayes, PhD candidate, Neuroscience graduate group, University of Pennsylvania (2012), Lisa Bottalico, PhD candidate Pharmacology Graduate Group, University of Pennsylvania (2011)

### **Supervisor of completed Master Theses**

2021-2023: Christos Gkougkousis, Thesis Title «Proteomic characterization of cardiomyocytes derived from human embryonic stem cells (hESCs). The role of nitric oxide signaling», I.I.P.P.S. Molecular and Cellular Biology and Biotechnology, University of Ioannina

### **Supervisor of ongoing Master Theses**

2023- current: Eleni Iordanidou, Title «Chemoselective methodologies to enrich for N-lysine acetylated proteins and peptides in biological mixtures» Post graduate studies program, Department of Chemistry, UoI

2022- current: Maria Symeonidou, Title « Chemoselective strategies to enrich for S-nitrosylated proteins and peptides in complex biological mixtures», I.I.P.P.S. Molecular and Cellular Biology and Biotechnology. *Public defense is pending*

2022- current: Maria-Lydia Zorzombokou, Title «Structural interrogation of S-nitrosylated proteins in mitochondria». Post graduate studies program, Department of Chemistry, UoI

### **Member of Advisory and Thesis committees**

#### **Advisory Committees**

- 2021-current: Stylianos Papadopoulos, PhD candidate, Department of Chemistry, UoI, Title “Spatio-temporal features and molecular mechanisms governing the establishment, progression and propagation of acute lung injury”
- 2017-2019: Laura C Sillers MD, Division of Neonatology, Department of Pediatrics, The Perelman School of Medicine, UPenn

#### **Member of PhD thesis examination committee**

- 2023: Epaminondas Tsagkogiannis, PhD candidate, Department of Chemistry, UoI, Thesis title “*Functional characterization of participating cleavage dioxygenases in protocatechoic acid metabolism in soil bacterium Pseudarthrobacter phenanthrenivorans Sph3: Their contribution to the metabolism of hydroxybenzoic acids*”
- 2020: Styliani Papadaki, PhD candidate, Department of Chemistry, UoI, Thesis title “*Study of the role of coagulation factors Xa and thrombin in atherothrombosis*”
- 2020: Sofia Sidiropoulou, PhD candidate, Department of Chemistry, UoI, Title “*Pleiotropic actions of platelet receptor agonists: underlying mechanisms and pathophysiological role*”

#### **Master examination committee**

- 2020: Stylianos Papadopoulos, Department of Chemistry, UoI, Thesis title “Expression, production and secretion of sPLA2-IIA in acute inflammation”

### **Awards**

2015: W.W. Smith Charitable Foundation Award for Heart Disease Research

2013: Top ten finalists for Vilcek Prize for Creative Promise in Biomedical Science

2013: New Investigator award, “Formation and Signaling by NO and Related Reactive Nitrogen Oxides” Gordon Research Conference, Ventura, CA

2011: Finalist of the 2011 CHOP Distinguished Research Trainee Award

2007: Post-doctoral fellowship funded by the National Institutes of Health (NIH)

2005: Post-doctoral fellowship co-funded by the European Union and the Hellenic Ministry of Education

2002: Oral presentation award, 3<sup>rd</sup> Hellenic Meeting of the Hellenic Society of Free Radical Research, Athens Greece

2002: Poster Presentation award, 56<sup>th</sup> Annual meeting of the Hellenic Society for Biochemistry and Molecular Biology

### **Academic Service**

#### **Active**

2023-current: Appointed as Education Ambassador of the Hellenic Society of Biochemistry and Molecular Biology in the Federation of European Biochemical Societies (FEBS)

2020-current: Member of the Coordinating Committee, I.I.P.P.S. Molecular and Cellular Biology and Biotechnology, University of Ioannina

2020-current: Member of the steering committee for the program of studies, I.I.P.P.S. Molecular and Cellular Biology and Biotechnology, University of Ioannina

#### **Completed**

2020-2022: Member of the Coordinating Committee, Post graduate studies program, Department of Chemistry, UoI

2016-2019: Regular Member of Institutional Animal Care and Use Committee (IACUC), CHOP-IR, Philadelphia, PA, USA

### **Patents**

Compositions and methods of the treatment of fatty acid metabolism disorders». International Application Number PCT/US2014048564

### **Memberships in Professionals and Scientific Societies**

2020-current: Member of the F1000 Faculty Society, Bioinorganic Chemistry Section.

2003-current: Member of the European Society for Free Radical Research

2002-current: Member of the Hellenic Society for Biochemistry and Molecular Biology

1998-current: Member of the Greek Society of Chemistry

### **Guest Editor**

Special Issue "Research on Fatty Acid Oxidation and Fatty Acid Oxidation Disorders" in Biomolecules

### **Member of Editorial Boards**

2023-current: Associate Editor, Frontiers of Physiology, Metabolic Physiology

2023-current: Editorial Board, Frontiers in Frontiers in Molecular Biosciences

### **Reviewer**

*Ad hoc* reviewer for:

*American Journal of Physiology-Endocrinology and Metabolism*

*Biochemical Journal*

*Biochimica et Biophysica Acta (BBA) General Subjects*

*Biochimie*

*Biomarkers*

*BMC Cardiovascular Disorders*

*FEBS letters*

*Free Radical Biology and Medicine*

*Free Radical Research*

*Frontiers in Cellular Biochemistry*  
*Frontiers in Oncology*  
*International Journal of Molecular Sciences*  
*Inorganic Chemistry Communications*  
*Journal of Lipids Research*  
*Metabolites*  
*Nitric Oxide*  
*Nutrients*  
*The Journal of Biological Chemistry*  
*The Journal of Proteomics*  
*PLOS One*  
*PLOS Pathogens*  
*Proteomics*

### **Grant Reviewer**

2020-current: The General Secretariat of Research and Technology

2020-current: Hellenic Foundation for Research and Innovation

2016-2020: The American Heart Association

### **Invited speaker in meetings**

- Nitric Oxide Gordon Research Conference, Molecular Mechanisms of Nitric Oxide Signaling in Health and Disease. Ventura Beach, California February 12-17, 2023
- The 71st National Conference of Hellenic Society of Biochemistry & Molecular Biology, Athens, Greece, November 26-28, 2021.
- The 11<sup>th</sup> International Meeting on Nitric Oxide in Chemistry, Biology and Therapeutic Applications. Chicago, IL, USA, May 10-13, 2020.
- Meeting of the Society For Free Radical Research (SFRR)-India, “Role and Management of Oxidative Stress in Human Disease”, Mumbai, India, February 12-15, 2020.
- The International Network for Fatty Acid Oxidation Research and Management (INFORM). Athens, Greece, September 2-3, 2018.
- Regulation of Fatty Acids Oxidation. Translating Mechanistic insights Into Therapeutics". Malcolm Feist Cardiovascular Diseases seminar series, Center for Cardiovascular Diseases and Sciences (CCDS), LSU Health Sciences Center. Shreveport, Louisiana, December, 2017
- Nitric Oxide Gordon Research Conference, Reactive Nitrogen Signaling: Mechanism to Medicine. Ventura Beach, California February 19-24, 2017
- The International Network for Fatty Acid Oxidation Research and Management (INFORM). Boston, MA, USA May 15-19, 2016
- The 4th Beijing International Conference of Neurology 2014, Beijing China, April 12-13, 2014
  
- First International Conference on S-nitrosylation in Oncology and Immunology. Dijon, France, February 2-3, 2012
- Meeting of the Society for Free Radical Research-Europe: Redox Biology and Micronutrients: From signaling to translation and back. Istanbul, Turkey, September 7-11, 2011

## **Publications**

### **Peer reviewed:**

1. Andreyev AY, Yang H, Doulias PT, Dolatabadi N, Zhang X, Luevanos M, Blanco M, Baal C, Putra I, Nakamura T, Ischiropoulos H, Tannenbaum SR, Lipton SA. (2024). Metabolic Bypass Rescues Aberrant S-nitrosylation-Induced TCA Cycle Inhibition and Synapse Loss in Alzheimer's Disease Human Neurons. *Adv Sci* 18:e2306469. Online ahead of print.
2. Doulias PT, Yang H, Andreyev AY, Dolatabadi N, Scott H, K Raspur C, Patel PR, Nakamura T, Tannenbaum SR, Ischiropoulos H, Lipton SA. (2023). S-Nitrosylation-mediated dysfunction of TCA cycle enzymes in synucleinopathy studied in postmortem human brains and hiPSC-derived neurons. *Cell Chem Biol* 30: 965-975
3. Khare S, Kim LC, Lobel G, Doulias PT, Ischiropoulos H, Nissim I, Keith B, Simon MC. (2021) ASS1 and ASL suppress growth in clear cell renal cell carcinoma via altered nitrogen metabolism. *Cancer Metab* 9: 40.
4. Lau B, Fazelinia H, Mohanty I, Raimo S, Tenopoulou M, Doulias PT, Ischiropoulos H. (2021). Endogenous S-nitrosocysteine proteomic inventories identify a core of proteins in heart metabolic pathways. *Redox Biol* 47: 102153
5. Doulias PT\*, Tenopoulou M, Zakopoulos I, Ischiropoulos H. (2021). Organic mercury solid phase chemoselective capture for proteomic identification of S-nitrosated proteins and peptides. *Nitric Oxide* 117: 1-6 \*Corresponding author
6. Zamani P, Proto EA, Wilson N, Fazelinia H, Ding H, Spruce LA, Davila A Jr, Hanff TC, Mazurek JA, Prenner SB, Desjardins B, Margulies KB, Kelly DP, Arany Z, Doulias PT, Elrod JW, Allen ME, McCormack SE, Schur GM, D'Aquila K, Kumar D, Thakuri D, Prabhakaran K, Langham MC, Poole DC, Seeholzer SH, Reddy R, Ischiropoulos H, Chirinos JA. (2021). Multimodality assessment of heart failure with preserved ejection fraction skeletal muscle reveals differences in the machinery of energy fuel metabolism. *ESC Heart Fail* 8: 2698-2712
7. Doulias PT, Nakamura T, Scott H, McKercher SR, Sultan A, Deal A, Albertolle M, Ischiropoulos H, Lipton SA (2021). TCA cycle metabolic compromise due to an aberrant S-nitrosoproteome in HIV-associated neurocognitive disorder with methamphetamine use. *J Neurovirol* J Neurovirol. 27: 379
8. Ramick MG, Kirkman DL, Stock JM, Muth BJ, Farquhar WB, Chirinos JA, Doulias PT, Ischiropoulos H, Edwards DG. (2021). The effect of dietary nitrate on exercise capacity in chronic kidney disease: a randomized controlled pilot study *Nitric Oxide - Biology and Chemistry* 106: 17-23
9. Umanah GKE, Ghasemi M, Yin X, Chang M, Kim JW, Zhang J, Ma E, Scarffe LA, Lee YI, Chen R, Tangella K, McNamara A, Abalde-Atristain L, Dar MA, Bennett S, Cortes M, Andrabi SA, Doulias PT, Ischiropoulos H, Dawson TM, Dawson VL. (2020). AMPA Receptor Surface Expression Is Regulated by S-Nitrosylation of Thorase and Transnitrosylation of NSF. *Cell Rep.* 33:108329
10. Vartika Mishra V, Diane B. Re DB, Le Verche V, Alvarez MJ, Alessandro Vasciaveo A, Jacquier A, Doulias PT, Greco TM, Nizzardo M, Papadimitriou D, Nagata T, Rinchetti P, Perez-Torres E, Politi K, Ikiz B, Clare K, Than ME, Corti S, Ischiropoulos H, Lotti F, Califano A, Przedborski S (2020). Systematic elucidation of neuron-astrocyte interaction in ALS using multi-modal integrated bioinformatics. *Nat. Commun.* 11: 5579-5598

11. Elshenawy S, Pinney SE, Stuart T, Doulias PT, Zura G, Parry S, Elovitz MA, Bennett MJ, Bansal A, Strauss JF III, Ischiropoulos H, Simmons RA (2020). The Metabolomic Signature of the Placenta in Spontaneous Preterm Birth. *Int J Mol Sci.* 21:1043
12. Doulias PT, Gould NS (2018). Analysis of Cysteine Post Translational Modifications Using Organic Mercury Resin. *Curr Protoc Protein Sci.* 94:e69
13. Doulias PT, Tenopoulou M, Nakamoto K, Berrios K, Zura G, Li C, Faust M, Yakovishina V, Evans P, Tan L, Bennett MJ, Snyder NW, Quinn WJ 3rd, Baur JA, Atochin DN, Huang PL, Ischiropoulos H (2018). Oral nitrite restores age-dependent phenotypes in eNOS-null mice. *JCI Insight.* 3. pii: 122156
14. Frawley ER, Karlinsey JE, Singhal A, Libby SJ, Doulias PT, Ischiropoulos H, Fang FC (2018). Nitric Oxide Disrupts Zinc Homeostasis in Salmonella enterica Serovar Typhimurium. *MBio.* 9: pii: e01040-18.
15. Guan D, Xiong Y, Borck PC, Jang C, Doulias PT, Papazyan R, Fang B, Jiang C, Zhang Y, Briggs ER, Hu W, Steger D, Ischiropoulos H, Rabinowitz JD, Lazar MA (2018). Diet-Induced Circadian Enhancer Remodeling Synchronizes Opposing Hepatic Lipid Metabolic Processes. *Cell* 174: 831-842
16. Urbano R., Karlinsey J.E. , Stephen J. Libby S.J., Doulias PT, Harry Ischiropoulos H., Helen I. Warheit-Niemi H.I., Liggitt D.H., Alexander R. Horswill A.R., Ferric C. Fang F.C. (2018). Host Nitric Oxide Disrupts Microbial Cell-to-Cell Communication to Inhibit Staphylococcal Virulence. *Cell Host and Microbe* 23:594-606
17. Lin W, Vann DR, Doulias PT, Wang T, Landesberg G, Li X, Ricciotti E, Scalia R, He M, Hand NJ, Daniel J. Rader DJ (2017). Hepatic *SLC39A8*/ZIP8 regulates whole-body manganese homeostasis and the activity of Mn-dependent enzymes. *J Clin Invest.* 127: 2407-2417
18. Chen F, WangY, RafikovR, Haigh S, Zhi WB, Kumar S, Doulias PT, Rafikova O, Pillich H, Chakraborty T, Lucas R, Verin AV, Catravas JD, She JX, Black SM, Fulton D Jr (2017). RhoA S-nitrosylation as a regulatory mechanism influencing endothelial barrier function in response to G<sup>+</sup>-bacterial toxins. *Biochem Pharmacol.* 127: 34-45
19. Zamani P, Tan VX, Soto-Calderon H, Beraun M, Brandimarto J, Trieu L, Varakantam S, Doulias PT, Townsend RR, Chittams J, Margulies KB, Cappola TP, Poole DC, Ischiropoulos H, Chirinos JA. (2017). Pharmacokinetics and Pharmacodynamics of Inorganic Nitrate in Heart Failure With Preserved Ejection Fraction. *Circ Res.* 120: 1151-1161
20. Chirinos JA, Akers SR, Trieu L, Ischiropoulos H, Doulias PT, Tariq A, Vassim I, Koppula MR, Syed AA, Soto-Calderon H, Townsend RR, Cappola TP, Margulies KB, Zamani P. (2016). Heart Failure, Left Ventricular Remodeling, and Circulating Nitric Oxide Metabolites. *J Am Heart Assoc.* 5: e004133
21. Zamani P, French B, Brandimarto JA, Doulias PT, Javaheri A, Chirinos JA, Margulies KB, Townsend RR, Sweitzer NK, Fang JC, Ischiropoulos H, Cappola TP. (2016). Effect of Heart Failure With Preserved Ejection Fraction on Nitric Oxide Metabolites. *Am J Cardiol.* 118: 1855-1860
22. Irie T, Sips PY, Kai S, Kida K, Ikeda K, Hirai S, Moazzami K, Jiramongkolchai P, Bloch DB, Doulias PT, Armoundas AA, Kaneki M, Ischiropoulos H, Kranias E, Bloch KD, Stamler J, Ichinose F. (2015). S-Nitrosylation of Calcium-Handling Proteins in Cardiac Adrenergic Signaling and Hypertrophy. *Circ Res.* 117: 793-803



23. Raju K, Doulias PT, Evans P, Krizman EN, Jackson JG, Horyn O, Daikhin Y, Nissim I, Yudkoff M, Nissim I, Sharp KA, Robinson MB, Ischiropoulos H. (2015). Regulation of brain glutamate metabolism by nitric oxide and S-nitrosylation. *Science Sig.*, 8:ra68 PMID: 26152695
24. Tenopoulou M, Chen J, Bastin J, Bennett MJ, Ischiropoulos H, Doulias PT\* (2015). Strategies for correcting very long chain acyl-CoA dehydrogenase deficiency. *J. Biol. Chem.* 290: 10486-10494 \* Corresponding author
25. Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, Cappola TP, Poole DC, Chirinos JA. (2015). The Effect of Inorganic Nitrate on Exercise Capacity in Heart Failure with Preserved Ejection Fraction. *Circulation* 131: 371-380
26. Henard CA, Tapscott T, Crawford MA, Husain M, Doulias PT, Porwollik S, Liu L, McClelland M, Ischiropoulos H, Vázquez-Torres A. (2014). The 4-cysteine zinc-finger motif of the RNA polymerase regulator DksA serves as a thiol switch for sensing oxidative and nitrosative stress. *Mol Microbiol.* 91:790-804.
27. Jaeckle Santos LJ, Li C, Doulias PT, Ischiropoulos H, Worthen GS, Simmons RA. (2014). Neutralizing Th2 inflammation in neonatal islets prevents  $\beta$ -cell failure in adult IUGR rats. *Diabetes* 63: 1672-1684
28. Doulias PT, Tenopoulou M, Greene JL, Raju K, Ischiropoulos H (2013). Nitric oxide regulates mitochondrial fatty acid metabolism through reversible protein S-nitrosylation. *Science Sig.*, 6:rs 1-7
29. Doulias PT\*, Tenopoulou M, Raju K, Spruce LA, Seeholzer SH, Ischiropoulos H (2013). Site specific identification of endogenous S-nitrosocysteine proteomes. *J Proteomics* 92: 195-203 \* Corresponding author
30. Lee YI, Giovinazzo D, Kang HC, Lee Y, Jeong JS, Doulias PT, Xie Z, Hu J, Ghasemi M, Ischiropoulos H, Qian J, Zhu H, Blackshaw S, Dawson VL, Dawson TM. (2013). Protein microarray characterization of the S-nitrosoproteome. *Mol Cell Proteomics* 13: 63-72
31. Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Abraham V, Doghramji L, Adappa ND, Palmer JN, Kennedy DW, Beauchamp GK, Doulias PT, Ischiropoulos H, Kreindler JL, Reed DR, Cohen NA (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest.* 122: 4145-4159
32. Thomson L, Tenopoulou M, Lightfoot R, Tsika E, Parastatidis I, Martinez M, Greco TM, Doulias PT, Wu Y, Tang WH, Hazen SL, Ischiropoulos H (2012). Immunoglobulins against Tyrosine Nitrated Epitopes in Coronary Artery Disease. *Circulation* 126:2392-2401
33. Doulias PT, Greene JL, Greco TM, Margarita Tenopoulou, Seeholzer SH, Dunbrack RL, Ischiropoulos H (2010) Structural profiling of endogenous S-nitrosocysteine residues reveals unique features that accommodate diverse mechanisms for protein S-nitrosylation. *Proc Natl Acad Sci U S A.* 107: 16958-16963.
34. Yang Z, Wang ZE, Doulias PT, Wei W, Ischiropoulos H, Locksley RM, Liu L (2010) Lymphocyte development requires S-nitrosoglutathione reductase. *J Immunol.* 185: 6664-6669
35. Doulias PT, Vlachou C, Boudouri C, Kanavaros P, Siamopoulos KC, Galaris D (2008) Flow cytometric estimation of 'labile iron pool' in human white blood cells reveals a positive association with ageing. *Free Radic Res* 42: 253-259

36. Doulias PT, Kotoglou P, Tenopoulou M, Keramisanou D, Tzavaras T, Brunk U, Galaris D, Angelidis C (2007) Involvement of heat shock protein-70 in the mechanism of hydrogen peroxide-induced DNA damage: The role of lysosomes and iron. *Free Radic Biol Med* 42: 567-577
37. Tenopoulou M, Kurz T, Doulias PT, Galaris D, Brunk UT (2007) Does the calcein-AM method assay the total cellular 'labile iron pool' or only a fraction of it? *Biochem J* 403: 261-266
38. Doulias PT, Nouis L, Zhu BZ, Frei B, Galaris D (2005) Protection by tropolones against H<sub>2</sub>O<sub>2</sub>- induced DNA damage and apoptosis in cultured Jurkat cells. *Free Radic Res* 39: 125-135
39. Tenopoulou M, Doulias PT, Barbouti A, Brunk U, Galaris D (2005) Role of compartmentalized redox-active iron in hydrogen peroxide-induced DNA damage and apoptosis. *Biochem J* 387: 703-710
40. Nouis L, Doulias PT, Aligiannis N, Bazios D, Agalias A, Galaris D, Mitakou S (2005) DNA protecting and genotoxic effects of olive oil related components in cells exposed to hydrogen peroxide. *Free Radic Res* 39: 787-795
41. Doulias PT, Christoforidis S, Brunk UT, Galaris D (2003) Endosomal and lysosomal effects of desferrioxamine: Protection of HeLa cells from hydrogen peroxide-induced DNA damage and induction of cell-cycle arrest. *Free Radic Biol Med* 35: 719-728
42. Barbouti A, Doulias PT, Nouis L, Tenopoulou M, Galaris D (2002) DNA damage and apoptosis in hydrogen peroxide-exposed Jurkat cells: Bolus addition versus continuous generation of H<sub>2</sub>O<sub>2</sub>. *Free Radic Biol Med* 33: 691-702
43. Tselepis A, Doulias PT, Lourida E, Glantzounis G, Tsimoyiannis E, Galaris D (2001). Trimetazidine protects low-density lipoproteins from oxidation and cultured cells exposed to H<sub>2</sub>O<sub>2</sub> from DNA damage. *Free Radic Biol Med* 30: 1357-136
44. Barbouti A, Doulias PT, Zhu BZ, Frei B, Galaris D (2001) Intracellular iron, but not copper, plays a critical role in hydrogen peroxide-induced DNA damage. *Free Radic Biol Med* 31: 490-498
45. Doulias PT, Barbouti A, Galaris D, Ischiropoulos H (2001) SIN-1-induced DNA damage in isolated human peripheral blood lymphocytes as assessed by single cell gel electrophoresis (comet assay). *Free Radic Biol Med* 30: 679-685

#### Peer reviewed reviews:

46. Tenopoulou M, Doulias PT\* (2020). Nitric oxide in the regulation of metabolism *F1000Research Volume 9, Article number 1190* \* Corresponding author
47. Gould N, Doulias PT, Tenopoulou M, Raju K, Ischiropoulos H (2013). Regulation of protein function and signaling by reversible cysteine S-nitrosylation. *Journal of Biological Chemistry minireview series on "Redox Sensing and Signaling"* 288: 26473-26479
48. Doulias PT; Raju K; Greene JL; Tenopoulou M; Ischiropoulos H (2013). Mass spectrometry-based identification of S-nitrosocysteine in vivo using organic mercury assisted enrichment. *Methods* 62: 165-170.
49. Raju K, Doulias PT, Tenopoulou M, Greene JL, Ischiropoulos H (2012) Strategies and Tools to Explore Protein S-Nitrosylation *BBA-General Subjects* 1820: 684-688

#### Book Chapters

1. M. Tenopoulou, P.-T. Doulias and D. Galaris (2008) Molecular mechanisms of H<sub>2</sub>O<sub>2</sub>-induced DNA damage. The action of desferrioxamine. Chapter 16 in “Chemistry and molecular aspects of drug design and action”, edited by E. A. Rekkas, P. N. Kourounakis and published by CRC Press, Taylor & Francis Group
2. P.-T. Doulias, M. Tenopoulou, D. Galaris and S. Christoforidis (2003). The Role of Lysosomal Iron in H<sub>2</sub>O<sub>2</sub>-induced DNA Damage, p 61-66. Meeting of the Society for Free Radical Research - European Section “Free Radicals and Oxidative Stress: Chemistry, Biochemistry and Pathophysiological Implications”, edited by D. Galaris and published by Monduzzi Editore International Proceedings Division

### **Abstracts and posters in meetings**

1. Symeonidou M, Gkougkousis C, Doulias PT. Chemoselective enrichment strategy to identify endogenous protein and site-specific S-nitrosylation. The 74th National Conference of Hellenic Society of Biochemistry & Molecular Biology, Athens, Greece, December 1-3, 2023
2. Gkougkousis C, Markou M, Murphy C, Doulias PT. Cardiomyocytes derived from differentiated embryonic stem cells as a model to study metabolism and nitric oxide signaling. The 72nd National Conference of Hellenic Society of Biochemistry & Molecular Biology, Patras, Greece, December 2-4, 2022
3. Tenopoulou M, Ischiropoulos H, Doulias PT. Novel therapeutics for fatty acid oxidation disorders. The 71st National Conference of Hellenic Society of Biochemistry & Molecular Biology, Athens, Greece, November 26-28, 2021
4. Doulias PT, Tenopoulou M, Nakamoto K, Berrios K, Zura G, Li C, Faust M, Yakovishina V, Evans P, Tan L, Bennett MJ, Snyder NW, Quinn WJ 3rd, Baur JA, Atochin DN, Huang PL, Ischiropoulos H. Restoration of age-dependent phenotypes by nitrite reveals a regulatory role for endothelial nitric oxide synthase/nitric oxide signaling in metabolic syndrome. In 10th International Conference on the Biology, Chemistry and Therapeutic Applications of Nitric Oxide, September 2018, Oxford, UK
5. Oh CK, Dolatabadi N, Soldner F, Jaenisch R, McClatchy DB, Diedrich JK, Yates JR 3rd, Ambasadhan R, Doulias PT, Ischiropoulos H, Nakamura T, Lipton SA. S-Nitrosylation of PINK1 (PINK-SNO) attenuates PINK1/Parkin-dependent mitophagy in hiPSC-derived neuron models of Parkinson’s Disease (PD): Toward assessment of the S-nitrosproteome in human PD in an unbiased manner by mass spectrometry. In 10th International Conference on the Biology, Chemistry and Therapeutic Applications of Nitric Oxide, September 2018, Oxford, UK
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### **Research Interests**

My research interests involve basic and translational research focusing on the following thematic areas.

#### Basic Research

We are interested in investigating the molecular mechanisms that regulate the biochemical pathway of mitochondrial fatty acid oxidation ( $\beta$ -oxidation). Beta oxidation represents the main pathway of fatty acid catabolism in mammalian cells. It utilizes long, medium and short chain fatty acids as a substrate for the production of acetyl coenzyme A (acetyl-CoA) and reducing equivalents. The heart, under physiological conditions, derives nearly 70% of ATP molecules that are required for contractile, ionic and other functions through the oxidation of long chain fatty acids (LC-FAs). Energy generation in exercising muscle is accomplished via the catabolism of fatty acid. Finally, the adaptive response to the physiological stress of fasting is achieved by the augmented rate of LC-FAO in the liver. The acetyl-CoA that is generated feeds into the TCA cycle whereas part of it is converted to ketone bodies. The latter are released into the circulation and are used to regenerate acetyl-CoA in the brain and kidney, two organs that cannot oxidize fatty acids.

Despite its physiological importance the molecular mechanisms regulating mFAO in health and disease remain incompletely understood.

**Our working hypothesis is that energy demands activate signaling cascades that intergrade on the enzymes participating to mFAO pathway in the form of post translational modifications (PTMs). These signals control enzymatic activity and coordinate pathway performance in accordance with energy demands.**

This hypothesis is tested in cells and animal models. Available models include mice with genetic deletion of the mitochondrial enzymes sirtuin-3 or sirtuin-5 as well as mice lacking the endothelial nitric oxide synthase (eNOS<sup>-/-</sup>). These genotypes display biochemical, metabolic and phenotypic changes consistent with impaired oxidation of LC-FAs. Sirt-3 is the major lysine deacetylase in the mitochondria. Its genetic deletion leads to hyperacetylation of metabolic enzymes including the proteins participating in mFAO. Sirtuin 5- (Sirt5) is the major lysine de-succinylase in the mitochondria. Succinylation is the posttranslational modification of lysine by succinate. Genetic deletion of Sirt5 in mice results in augmented levels of protein succinylation in mitochondria. Again, metabolic enzymes and specifically proteins participating in the mFAO pathway display augmented succinylation in the absence of Sirt5. Our published work in eNOS<sup>-/-</sup> mice documents reduced levels of NO and protein S-nitrosylation in the circulation and in several organs including the heart, liver and skeletal muscle. S-nitrosylation is a selective and reversible NO-derived PTM occurring on cysteine residues. We have also generated a mouse model harboring the Cys238A mutation on very long chain acyl-CoA dehydrogenase (VLCAF), the first enzyme in the mFOA pathway. Published work documents that cysteine 238 is necessary for the regulation of enzymatic activity by S-nitrosylation. This model will facilitate our understanding regarding the coordinated regulation of enzyme activity by PTMs.

Finally, the functional consequences of PTMs on enzymatic activity will be investigated in cell models using site directed mutagenesis.

#### Translational Research

This research area focused on long chain fatty acid oxidation disorders (LC-FAODs), a family of rare inherited autosomal recessive diseases that present with cardiomyopathy, intermittent muscle breakdown and liver failure. Treatments for LC-FAODs have largely involved nutritional and symptomatic management, thus *there is an unmet need to develop therapies for these inborn errors of metabolism*. The prevailing mechanism for the clinical phenotypes relates to the insufficient production of acetyl-CoA from the oxidation of LC-FAs leading to depletion of

tricarboxylic acid cycle (TCA) intermediates and inadequate generation of ATP to meet metabolic demands.

We have conceptualized an innovative therapeutic strategy towards LC-FAODs. Our working hypothesis stems from the discovery that nitric oxide signaling regulates the mFAO pathway through selective and reversible post-translational modification of cysteine residues to form S-nitrosocysteine. We have documented that: (1) S-nitrosylation of cysteine residue 237 in human (238 in mouse) in VLCAD lowered the KM by 5-fold and increased the catalytic efficiency by 29-fold<sup>8</sup>. (2) Long term administration of bioactive NO in eNOS<sup>-/-</sup> mice restored the capacity to metabolize fatty acids and metabolic homeostasis<sup>10</sup>. (3) Pharmacological delivery of nitric oxide to human VLCAD-deficient fibroblasts restored the catalytic efficiency of the mutant protein and corrected the acylcarnitine profile, a diagnostic marker of the disease<sup>9</sup>. (3) Treatment of VLCAD-deficient cells with 2-mononitrate-1,3-diheptanoin (MNDH), an innovative hybrid molecule that simultaneously delivers NO and metabolic substrate in the form of heptanoic acid, restored the catalytic efficiency of mutant VLCAD and increased the rate of palmitate oxidation.

Therefore, based on this evidence as well as the knowledge derived from the use of nitric oxide medications in humans, we propose to test nitric oxide (NO) as a potential therapeutic for LC-FA oxidation disorders. Currently, we are trying to achieve the following goals. (1) *Comprehensive evaluation and characterization of small molecules to augment nitric oxide signaling.* We are testing existing FDA approved NO-delivering molecules as well as patent protected hybrid compositions that simultaneously deliver NO and metabolic substrate. (2) *Determine how augmented NO signaling restores LC-FAO and corrects the metabolic insufficiency in common LC-FAODs.* The compounds with favorable properties are tested in cells from patients with LC-FAODs, (3) *Test the efficacy of NO signaling to correct VLCAD-deficiency in vivo.* VLCAD-deficiency represents the most common LC-FAOD presenting with the most severe and life-threatening symptoms. These studies involve the generation of a mouse model expressing human VLCAD harboring the pathologic mutations V283A/V283A and its comprehensive biochemical, metabolic, and phenotypic assessment.