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SECTION A – UNGERGRADUATE STUDIES & GRANTS

1996 – 2000: Undergraduate studies in the Department of Chemistry, University of Crete (graduation grade: **8.87/10**)

Grants: (1) Entry Exam IKY Award - Scholarship (1996), (2) 1st, 2nd, 3^d and 4th year IKY Scholarship for Excellence (1996-2000), (3) 3^d year Award for Excellence (Municipality of Heraklion), (4) Award of Excellence in Graduation, University of Crete – Department of Chemistry (2000).

SECTION B – (POST)GRADUATE STUDIES & GRANTS

2006 – 2010: **Research Collaborator** under fellowship (GRID COMPUTATIONAL CHEMISTRY – GRID-COMP-CHEM – Marie-Curie Host Fellowship for the Transfer of Knowledge **EU ToK grant No. MTKD-CT-2005-029583**) in the Institute of Electronic Structure and Laser of the Foundation for Research and Technology-Hellas (**IESL-FORTH**).

2008 – 2009: **Visiting Postdoc** at Barcelona Supercomputing Center (**BSC**), Life Sciences department (GRID COMPUTATIONAL CHEMISTRY – GRID-COMP-CHEM – Marie-Curie Host Fellowship for the Transfer of Knowledge **EU ToK grant No. MTKD-CT-2005-029583 & HPC-Europa2 Scholarship**).

2005: **Visiting Ph.D Grant-in-Aid for Scientific research (Grant-in-Aid for Specially Promoted Research) FY2005. Okazaki Institute** for Integrative Bioscience, National Institutes of Natural Sciences Supercomputing Center, **Okazaki, Aichi /Japan**.

2000 – 2006: **M.Sc. & Ph.D, Theoretical and Computational Biophysics and Biochemistry**, Department of Chemistry, University of Crete –

Msc Title: “The mechanism of O-O cleavage in Cytochrome c Oxidase as revealed by Density Functional Theory and *resonance* Raman”.

Ph.D Title: “Bioenergetic Mechanisms of O₂ and NO by Density Functional Theory”.

(1) **IKY Scholarship** for entry to the Postgraduate Programme (2001-2002), (2) **Maria Manasaki Scholarship** (2001-2002, 2002-2003)

(2) Project title: **Dynamics and reactivity of enzymatic processes involving heme-copper aa3 oxidase from *P. denitrificans*: Spectroscopy and theoretical studies**
Financial entity: **Greek Ministry of Education - Pythagoras I (2006)**

(3) Project title: **Bioenergetic mechanisms of O₂ and NO as revealed by density functional theory**
Financial entity: **Greek Ministry of Education / E.U. Hrakleitos (2002-2005)**

(4) Project title: **Structural Analysis of nitric oxide reductase (nor)**
Financial entity: **Greek Ministry of Education, General Secretariat for Scientific Research (2000-2001)**

Postgraduate Courses during MSc/ Ph.D (grade: 9.75/10):

(a) Special Topics in Biochemistry, (b) Pharmaceutical Chemistry, (c) IR/Raman Spectroscopy for Biological Molecules, (d) Computational Environmental Chemistry, (e) Multidimensional NMR Spectroscopy, (f) Vibrational Spectroscopy

SECTION C – POSITIONS/ EMPLOYMENT & GRANTS

Jan 2015 – present:

Assistant Professor, Cyprus University of Technology, Department of Environmental Science and technology.

1. HPC-Europa3 Transnational Access programme (HPC17K4FRZ) for Sabbatical Leave (2018). Department of Physics & Earth Sciences (Biophysics Group), Jacobs University, Bremen, Germany.
2. Cyprus Research Promotion Foundation project **POST-DOC/0916/0049** “*Triggering Photoprotection in Photosystem II Antenna by Molecular Simulations and Raman Spectroscopy*” (~160.000 Euros), as coordinator.
3. Cy-Tera-LinkSCEEM, PRACE HPC Production Grants ~5mn total cpu standard core-hours for projects: pro14b114s1, pro14b105s1 and pro15a113s1, pro15b104, pro16a105, pro16b103, pro17a103, pro17b101, PRACE DECI-13 LHCFlex, and PRACE DECI-14 AIMDPSII as coordinator.

4. COST Action CM1401: Our Astro-Chemistry History. Management Committee MC member (start April 2015).
5. Erasmus Grant Mobility for Training (visitor to the Academy of Sciences of the Czech Republic, Institute of Organic Chemistry and Biochemistry, Prague). May 2015.

Dec 2010 – Dec 2014:

Lecturer, Cyprus University of Technology, Department of Environmental Science and technology

1. Startup Grant Cyprus University of Technology (40.000 €)
2. "Activation and reduction of Nitric Oxide (NO) to laughing gas (N₂O) by Nitric Oxide Reductase (NOR) and heme-copper *cbb*₃ oxidase (financial entity: Cyprus Research Promotion Foundation €133.181. Technology/ΘΕΠΙΣ/0609/05 (BE): (2011-2013) as a collaborator.
3. Cy-Tera & LinkSCEEM HPC Preparatory Access and Production Grants (860.000 total cpu standard core-hours, for projects: LinkSCEEM Isprob104s1, Ispro13a121s1, pro13b113s1, and pro14a120) as coordinator.
4. The Deutscher Akademischer Austauschdienst (DAAD)/ German Academic Exchange Service Grant for ASEMUNDUS Networking Programme (Collaboration between Universities in Asia-Europe).
5. COST Action ES1206: Advanced Global Navigation Satellite Systems tropospheric products for monitoring severe weather events and climate (GNSS4SWEC) – Management Committee & WG2 member (terminated April 2015)

SECTION D – SCIENTIFIC SOCIETIES MEMBERSHIP

Active -

- (1) American Chemical Society (ACS) Member
- (2) Biophysical Society (BPS) Member
- (3) Cytera-EM HPC Resource Allocation Committee Member (The Cyprus Institute)
- (4) Supercomputing Facility User Forum Board (The Cyprus Institute)

Past -

- (1) Royal Society of Chemistry (RSC) Affiliate Member (2014-2016)
- (2) American Geophysical Union (AGU) Member (2016)

SECTION E – TEACHING ACTIVITIES AND ADMINISTRATIVE DUTIES

Teaching Assistant (MSc, Ph.D, postdoc: 2000-2008)

University of Crete, Department of Chemistry

(a) Laboratory of Inorganic Chemistry II, 2001, (b) Laboratory of General Chemistry I, 2001, (c) General Chemistry I, 2002, (d) Biochemistry I, 2003, 2006 & 2008.

Independent Teaching (Lecturer, Assistant Professor: 2011-present)

Cyprus University of Technology, Department of Environmental Science and Technology

In the recent years (2011-2017), I have/ had independent **teaching duties** for the following courses in the Department of Environmental Science and Technology in Cyprus University of Technology in **postgraduate**: (1) **Computational Environmental Chemistry, Biochemistry and Biology** (Spring Semesters 2012-2017), (2) **Special Topics in Environmental Bioscience** (Autumn Semester 2014), and **undergraduate levels**: (3) **Environmental Computational Modeling & Introduction to MATLAB** (Spring Semesters 2011-present) (4) **Dispersion Modeling** (Autumn Semesters 2011-2017), (5) **Atmospheric Physics – Meteorology** (Autumn Semester 2011) and (6) **Thermodynamics** (Spring Semesters 2011-2012).

Academic & Administrative Duties:

Cyprus University of Technology, Department of Environmental Science and Technology

1. The **Senatorial Committee of Information Systems and Technology (IST)** (2012-present)
 - (a) *Reviews on the annual budgetary submissions from IST*
 - (b) *IdM (IAM) Project Committee for the implementation of the “Identity Access Management” scheme into the Cyprus University of Technology Information Systems & Technology (IST)*
 - (c) *Microsoft Software for Students Project Committee (IST)*
 - (d) *Committee for the Internet User Access Rights and Policies for Cyprus University of Technology*
2. The Departmental **Postgraduate (2011-present) & Undergraduate Committees** (2017-present)
3. The Departmental **Committee on Quality Assurance** (2011-present)

4. The *Ad hoc* **ECTS label Committee** of the Cyprus University of Technology (2014)
5. The *Ad hoc* **committee for the Open University** (2016-present)
6. The **committee of the Pancyprian Examinations** for admission to Higher Education Institutions of Cyprus and Greece (2014)
7. Member of the **election committee** for the University Academic Board Members (2012)

SECTION F – RESEARCH ACTIVITIES

I am the leader of the Computational Environmental Modeling (CEM) Group in the Dept. Environmental Science and Technology (Cyprus University of Technology). My independent research focuses on studies of large biological and small gaseous molecules involved in processes of biological and environmental significance. Among others, my research interests include the biophysics and biochemistry behind the structure and functionality of heme proteins, e.g. the interaction of myoglobin (Mb) and Cytochrome *c* Oxidase (CcO) with atmospheric gases (CO_x, NO_x). Insight into their *in vivo* behavior and possible environmental sensor capabilities is obtained. My studies on Photosystem II (PSII) are related to the mechanism of excess energy dissipation (Non-Photochemical Quenching or NPQ) and the plant photoprotection process that is highly relevant to a changing climate in terms of the production of tolerant plants, the O₂ evolution and CO₂ fixation. On the other hand, my studies on water dynamics (hydration), viewed from the physical chemistry aspect, add to the multi-disciplinary nature of my research and will be used in the future as the basis for protein-hydration dynamics.

The ever-growing environmental concerns push researchers to identify renewable energy resources and satisfy the demand of an increasing global population. In nature, the life-sustaining oxygen cycle: $O_2 + 4e^- + 4H^+ \Leftrightarrow 2H_2O$ is driven by the processes of photosynthesis and cellular respiration (oxidative phosphorylation). In the latter two processes energy is released-harvested from bond cleavage and con-

sumed-stored in bond forming processes. Scientists are constantly targeting these processes in cutting-edge research in order to design synthetic systems that resemble their key components, duplicate their function and give fundamental insights into mechanisms of action. Moreover, especially for photosynthesis and Photosystem-II inspired models, this can potentially lead to applications in artificial and more efficient solar energy harvesting. It can also be related, in a timely manner, with the engineering of tolerant plants in a changing planetary climate (light stress, heat waves, increase of salinity in a deteriorating water quality). However, the drawback for an efficient photosynthetic process lies on the fact that under excess light conditions, organisms activate their photoprotection and convert a significant amount of photonic energy into heat. Apart from the recent studies on CP29, and the major LHCII, from my group, related all-atom simulations are lacking in the literature that can provide crucial protein conformational changes vital to photoprotective biochemistry and also targets for protein or inhibitor engineering. Cutting-edge research projects on Photosynthesis and Oxidative Phosphorylation will provide on one hand a deeper understanding of the molecular photoprotective cycle at all-atom resolution that is still obscure to eliminate the aforementioned drawback, and on the other hand, a complete biochemical oxygen cycle, with the mechanistic similarities of how redox active transition metal ions (Fe, Cu, Mn) make or break the strong O–O bond. This makes Cytochrome *c* Oxidase (CcO) also an extremely important enzyme. My dual insight in combining both computational and experimental biochemistry, biophysics and biological chemistry makes me one of the most suitable researchers who could handle successfully this demanding project and It is important that Eastern Mediterranean is also involved in this research.

I am reviewer for ACS Physical Chemistry Journals, RSC Phys. Chem. Chem. Phys., RSC Advances, Springer J. Molecular Modeling, OMICS Chemistry Journals, Biochemistry & Pharmacology, and for proposals to High Performance Computing (HPC) facilities (LinkSCEEM, CyTera-EM, GRNET HPC, CSCS-Swiss National Supercomputing Centre). I belong to the editorial board of the Chemistry of Compounds Journal (Verizona Publisher) and the SRL Biology Journals (SciRes Literature).

SECTION G – INTERNATIONAL COLLABORATIONS (OLD & CURRENT)

Prof. Farantos Stavros, Dept. of Chemistry, University of Crete & Foundation for Research and Technology-Hellas

Prof. Teizo Kitagawa, Specially Appointed Professor; Graduate School of Life Science, University of Hyogo

Prof. Guallar Victor, ICREA Research Professor at Barcelona Supercomputing Center - Centro Nacional de Supercomputación (BSC-CNS). Life & Medical Sciences .

Prof. Alexander Ruban, School of Biological and Chemical Sciences, Queen Mary, University of London

Prof. Ulrich Kleinekathöfer, Computational Physics and Biophysics Group, Jacobs University Bremen gGmbH, Department of Physics & Earth Sciences Focus Area Health

SECTION H – MENTORING OF STUDENTS

Undergraduate Diploma Theses (Bachelors' degree)

Completed (9): Charalambous Fevronia, Ioannou Neofyta, Panagiotou Fostira, Nearchou Irene, Demetriou D Constantinos, Georgiou A Georgia, Eleftheriou Antonis, Vorka Flora, Charalambous Andreas, Palazis K. Andreas, Katsoni Panagiota

Postgraduate Diploma Theses (Master's degree)

Completed (3): Charalambous Fevronia, Ioannou Neofyta, Papadatos Sotirios

Doctoral Theses/ Candidates (Ph.D degree):

Current (3): Salameh Anastasia, Papadatos Sotirios, Constantinou Anastasia

Participation in Examiner's Board for diploma theses (under/post-graduate): 20

SECTION I – LIST OF PUBLICATIONS

PUBLICATIONS IN INTERNATIONAL PEER-REVIEWED JOURNALS

*Corresponding Author

01. **V. Daskalakis V*** *Protein-Protein Interactions within Photosystem II under Photo-protection: The Synergy between CP29 Minor Antenna, Subunit S (PsbS) and Zeaxanthin at all-atom resolution (2018) Phys. Chem. Chem. Phys.* 20, 11843 – 11855.
02. **Daskalakis V***, and Papadatos S *The Photosystem II Subunit S under Stress (2017) Biophys. J. (Cell)* 113 (11), 2364-2372.
03. Papadatos S, Charalambous C A, and **Daskalakis V*** *A pathway for protective quenching in antenna proteins of Photosystem II (2017) Scientific Reports (NPG)* 7, 2523. doi: 10.1038/s41598-017-02892-w.
04. Ioannou A, **Daskalakis V**, and Varotsis C* *Detection of Maillard reaction products by a coupled HPLC-Fraction collector technique and FTIR characterization of Cu(II)-complexation with the isolated species (2017) J. Mol. Struct.* 1141, 634–642.
05. Ioannou A, Lambrou A, **Daskalakis V*** and Pinakoulaki E* *Coupling of helix E-F motion with the O-nitrito and 2-nitrovinyl coordination in myoglobin (2017) Biophys. Chem.* 221, 10-16.
06. Ioannou A, Lambrou A, **Daskalakis V** and Pinakoulaki E* *Nitrite coordination in myoglobin (2017) J. Inorg. Biochemistry (JIB)*, 166, 49-54.
07. Ioannidis NE, Papadatos S, and **Daskalakis V*** *Energizing the Light Harvesting Antenna: Insight from CP29 (2016) BBA – Bioenergetics*, 1857 (10), 1643-1650.
08. Salameh A, Vorka, F, and **Daskalakis V*** *Correlation Between Surface Tension and the Bulk Dynamics in Salty Atmospheric Aquatic Droplets (2016) J. Phys. Chem. C*, 120 (21), 11508-11518
09. **Daskalakis V**, Ohta T, Kitagawa T, Varotsis C* *Structure and properties of the catalytic site of nitric oxide reductase at ambient temperature (2015) Biochim. Biophys. Acta – Bioenergetics*, 1847(10), 1240–1244.
10. **Daskalakis V***, Charalambous F, Demetriou DC, Georgiou AG *Surface-Active Organic Matter induces salt morphology transitions during new atmospheric particle formation and growth (2015) RSC Adv.*, 5, 63240–63251.

- 11. Daskalakis V***, Charalambous F, Panagiotou F, Nearchou I *Effects of Surface Activity on Carbon Dioxide Nucleation in Atmospheric Wet Aerosols: A Molecular Dynamics Study* (2014) *Phys. Chem. Chem. Phys.*, 16: 23723-23734.
- 12. Daskalakis V***, Hadjicharalambous M *Hexagonal Ice Stability and Growth in the presence of Glyoxal and Secondary Organic Aerosols* (2014) *Phys. Chem. Chem. Phys.* 16(33): 17799-17810.
- 13.** Pinakoulaki E, **Daskalakis V**, Ohta T, Richter O M, Budiman K, Kitagawa T, Ludwig B, Varotsis C* *The protein effect in the structure of two ferryl-oxo intermediates at the same oxidation level in the heme-copper binuclear center of cytochrome c oxidase.* (2013) *J. Biol. Chem.* 288(28): 20261-20266.
- 14.** Porrini M, **Daskalakis V**, Farantos SC* *Exploring the Topography of Free Energy Surfaces and Kinetics of Cytochrome c Oxidases interacting with small ligands* (2012) *RSC Adv.* 2: 5828 - 5836.
- 15.** Kampanarakis A, Farantos SC*, **Daskalakis V** and Varotsis C *Non-Linear Vibrational Modes in Biomolecules: a periodic orbits description.* (2012) *Chem. Phys. Elsevier*, 399: 258–263.
- 16.** Pinakoulaki E, **Daskalakis V** and Varotsis C* (All authors have contributed equally to this work) *The origin of the Fe^{IV}=O intermediates in cytochrome aa₃ oxidase.* (2012) *BBA Bioenergetics*, 1817: 552-557.
- 17. Daskalakis V**, Farantos SC, Guallar V, Varotsis C* *Regulation of Electron and Proton Transfer by the Protein Matrix of Cytochrome c Oxidase.* (2011) *J. Phys. Chem. B*, 115(13): 3648-3655.
- 18. Daskalakis V***, Farantos SC, Guallar V, Varotsis C *Vibrational Resonances and Cu_B displacement controlled by proton motion in Cytochrome c Oxidase,* (2010) *J. Phys. Chem. B*, 114(2): 1136-1143.
- 19. Daskalakis V**, Varotsis C* *Binding and Docking Interactions of NO, CO and O₂ in Heme Proteins as Probed by Density Functional Theory, Review for the Special Issue on "Application of Density Functional Theory in Chemical Reactions".* (2009) *Int. J. Mol. Sci.*, 10: 4137-4156.
- 20.** Porrini M*, **Daskalakis V**, Farantos SC, Varotsis C *Heme Cavity Dynamics of Photodissociated CO from ba₃-Cytochrome c Oxidase: The Role of Ring-D Propionate,* (2009) *J. Phys. Chem. B*, 113(35): 12129-12135.
- 21. Daskalakis V**, Farantos SC*, Varotsis C *Assigning vibrational spectra of ferryl-oxo intermediates of cytochrome c oxidase by periodic orbits and Molecular Dynamics,* (2008) *J. Am. Chem. Soc* 130(37): 12385-12393.

22. Daskalakis V, Pinakoulaki E, Stavrakis S, Varotsis C* *Probing the environment of Cu_B in heme-copper oxidases. (2007) J. Phys. Chem. B* 111: 10502-10509.

23. Pinakoulaki E, Yoshimura H, **Daskalakis V**, Yoshioka S, Aono S, Varotsis C* *Two ligand binding sites in the O₂-sensing signal transducer HemAT: Implications for ligand Recognition/ Discrimination and signaling. (2006) Proc. Natl. Acad. Sci. USA* 103: 14796-14801.

24. Pinakoulaki E, Koutsoupakis C, Stavrakis S, Marialena A, Papadopoulos G, **Daskalakis V**, Varotsis C* *Structural dynamics of Heme-copper oxidases and Nitric oxide reductases: Time-Resolved step-scan FTIR and Time-resolved Resonance Raman studies. (2005) J. Raman Spec.* 36: 337-349.

25. Koutsoupakis C, Pinakoulaki E, Stavrakis S, **Daskalakis V**, Varotsis C* *Time-resolved step-scan Fourier transform infrared investigation of heme-copper oxidases: implications for O₂ input and H₂O/H⁺ output channels. (2004) Bioch. Biophys. Acta* 1655: 347-352.

CHAPTERS IN BOOKS, REVIEW ARTICLES

01. Salameh A, and **Daskalakis V*** *Atmospheric Ice Nucleation by Glassy Organic Compounds: A Review (2017), Chem. Comp. J* 1(1): 13-23.

02. Daskalakis V, Varotsis C* *Probing the Action of Cytochrome c Oxidase (2014) The Structural Basis of Biological Energy Generation, Advances in Photosynthesis and Respiration*, 39 (10): SBN 978-94-017-8742-0.

03. Daskalakis V*, Farantos S C, Varotsis C *Tuning heme functionality: the cases of Cytochrome c Oxidase and Myoglobin Oxidation (2012), LNCS – Springer-Verlag Berlin, Heidelberg*, p. 304-315. ISBN: 978-3-642-31124-6.

04. Daskalakis V, Giatromanolakis M, Porrini M, Farantos, SC* and Gervasi O *Grid Computing Multiple Shooting Algorithms for Extended Phase Space Sampling and Long Time Propagation in Molecular Dynamics. Computer Physics, Chapter 4 pp. 1-18, 2011 © Nova Science Publishers, Inc. Editors: B. S. Doherty, A. N. Molloy.* ISBN 978-1-61324-790-7.

PUBLICATIONS IN REFERRED PROCEEDINGS OF INTERNATIONAL CONFERENCES

01. Daskalakis V*, Farantos SC, Varotsis C *Protein Dynamics and Spectroscopy for Ferryl Intermediate of Cytochrome c Oxidase: A Molecular Dynamics Approach. (2007) AIP Computation in Modern Science and Engineering, Proc.* 963(2): 31-34.

SECTION J – PARTICIPATIONS IN CONFERENCES-WORKSHOPS-SEMINARS

1. **Daskalakis V.** 62nd Annual Meeting Biophysical Society, **S(T)IMULATING THE INTERPLAY BETWEEN PHOTOSYSTEM II PROTEINS AND THE XANTHOPHYLL CYCLE IN PHOTOPROTECTION**, San Francisco, USA, 17-21 February 2018
2. **Daskalakis V.** 3^d EMN Meeting on Computation and Theory – Molecular Dynamics and Its Applications, **The effect of proton and potential gradients on integral membrane proteins: Insights from Molecular Dynamics simulations**, Dubai UAE, 6-10 November 2017 (Invited Talk)
3. **Daskalakis V**, Papadatos S. 42nd FEBS Congress (from molecules to cells and back) – **The Photosystem II Subunit S Dynamics under Stress**, Jerusalem Israel, 10-14 September 2017 (Poster).
4. **Daskalakis V**, Salameh A, AGU 2016 Fall Meeting – **Correlation Between Surface Tension and the Bulk Dynamics in Salty Atmospheric Aquatic Droplets**, San Francisco USA, 12-16 December 2016 (Poster).
5. **Daskalakis V**, Ioannidis NE, Papadatos S. 79th Harden Conference – Oxygen Evolution and Reduction – Common Principles: **Tuning of a conformational change in CP29 from spinach: from Light Harvesting to Photoprotection**, Innsbruck Austria, 16-20 April 2016 (Poster+Talk).
6. **Daskalakis V** and Pinakoulaki E. New Biological Frontiers Illuminated by Molecular Sensors and Actuators meeting being held in Taipei, Taiwan, June 28 – July 1, 2015. Abstract titled “Globin Nitrite Heme Fe-O-N=O/ 2-Nitrovinyl Species: Implications for Myoglobin Helices Dynamics”, fixed-participants meeting.
7. Organizational Committee “12th Greece-Cyprus Conference on Chemistry 2015”, 8-10 May 2015, Thessaloniki Greece
8. **Daskalakis V**, Hadjicharalambous M, Charalambous F ***Ice 1_h crystal stability and growth in the presence of glyoxal Secondary Organic Aerosol and Carbon Dioxide.*** 13th Quadrennial IGACGP Symposium 13th IGAC Science Conference on Atmospheric Chemistry (IGACGP, IGAC 2014), Brazil, Natal, 22-26 September, 2014.
9. **Daskalakis V**, Hadjicharalambous M ***Probing the Effects of Atmospheric Pollutants on Ice Nucleation.*** International Conference on Scientific Computing 2013 (CSC 2013), Paphos, Cyprus, 3 - 6 December 2013. Invited Speaker.

10. **Daskalakis V**, Varotsis C **The structure of the Hyponitrite in Nitric Oxide Reductase (NOR)**. Biophysical Society (BPS) 57th Annual Meeting in Philadelphia, Pennsylvania, February 2-6, 2013.
11. **Daskalakis V**, ASEMUNDUS Networking seminar in Korea. Erasmus Mundus programme to enhance quality in higher education through scholarships and academic cooperation between Europe and the rest of the world. Seoul, Korea, May 15-16, 2012.
12. **Daskalakis V**, Farantos S C, Varotsis C **Tuning heme functionality: the cases of Cytochrome c Oxidase and Myoglobin Oxidation**, 12th International Conference on Computational Science and Applications (ICCSA 2012), June 18-21, Salvador de Bahia, Brazil, 2012. (*Best paper Award*)
13. **Daskalakis V**, Varotsis C **Probing the effect of the proximal and distal to the heme a_3 environments in the Cytochrome c Oxidase dioxygen reaction**, 15th International Conference on Biological Chemistry (ICBIC15), 7-12 August, Vancouver, Canada, 2011.
14. CD-ADAPCO, STAR-CCM+ Training School, 10-12 May 2011, London, UK.
15. **Daskalakis V**, Farantos S C, Guallar V, Varotsis C **QM/MM Calculations on Cytochrome c Oxidase: Probing of electron and proton pump coupling**, TAM 2010 MEETING, Helsinki 15-17 June 2010.
16. **Expanding the Frontiers of Molecular Dynamics Simulations in Biology**, 23-25 November, Institut d'Estudis Catalans, Barcelona – Spain, 2009 (Joint BSC - IRB Barcelona Conference).
17. Aug. 31 – Sept. 5 2009: Summer School on Simulation Approaches to Problems in Molecular and Cellular Biology, Miramar Palace, San Sebastian, Spain. **Cecam** (Centre Europeen de Calcul Atomique et Moleculaire), *under Psi-K scholarship*.
18. **Daskalakis V**, Farantos S C, Guallar V, Varotsis C **Towards the Understanding of His411-Fe^{IV}=O Spectroscopic Properties in Ferryl Intermediate of Cytochrome c Oxidase + O₂ Reaction: A Theoretical QM/MM, MD Approach**, 14th International Conference on Biological Inorganic Chemistry (ICBIC14), 25-30 July, Nagoya, Japan, 2009. **SBIC (The Society of Biological Inorganic Chemistry) Poster Award for outstanding contribution**.
19. **Porrini M**, **Daskalakis V**, Farantos SC, Varotsis C **Heme cavity dynamics of photo-dissociated CO from ba_3 -cytochrome c oxidase: The role of ring-D propionate**, 10th Congress in Chemistry Greece – Cyprus, 2-4 July Heraklion, Greece, 2009.

20. a. **Daskalakis V**, Farantos C S, Varotsis C **Assigning Vibrational Spectra of Ferryl-Oxo Intermediates of Cytochrome c Oxidase by Periodic Orbits and Molecular Dynamics**,

b. **Daskalakis V**, Varotis C **Cytochrome c Oxidase + O₂ reaction intermediates as probed by Density Functional Theory: The Proximal and Distal to heme α_3 effects**,

c. **Daskalakis V**, Varotsis C **Theoretical Studies of Metal-NO_x species, isolated and in aqueous solutions: Raman bands**, 10th Congress in Chemistry Greece – Cyprus, 2-4 July Heraklion, Greece, 2009.

21. 15-18 Sept. 2008: **Cost Training School On Molecular And Material Science Grid Applications** - Trieste, Italy.

22. **Gervasi O**, Farantos S C, **Daskalakis V**, Giatromanolakis M **The Study of cytochrome c Oxidase on the EGEE Grid**, 3d EGEE User Forum, Clemon-Ferrand, France, 2008.

23. **Daskalakis V**, Giatromanolakis M, **Farantos SC**, Gerbasi O **EGEE: Applications in Classical and Quantum Molecular Dynamics**, First Hellas Grid User Forum, Athens, Greece, 2007.

24. **Daskalakis V**, Farantos S, Varotsis C **Protein dynamics and spectroscopy for ferryl intermediate of Cytochrome c Oxidase: A molecular dynamics approach**, International Conference of Computational Methods in Sciences and Engineering (**ICCMSE 2007**), Corfu, Greece, 2007. (Award for Best Presentation, by the Organizing Committee of ICCMSE)

Publication: **Protein Dynamics and Spectroscopy for Ferryl Intermediate of Cytochrome c Oxidase: A Molecular Dynamics Approach**, *AIP Computation in Modern Science and Engineering, Proc.* 963(2): 31-34 (2007).

25. Pinakoulaki E, Ohta T, **Daskalakis V**, Aggelaki M, Kitagawa T, Ludwig B, **Varotsis C** **Diogen activation and bond cleavage in cell respiration as probed by resonance raman spectroscopy**, The second International Symposium on Biomolecular Chemistry (**ISBC2006**), Konan Fiber, Fiber, Konan University, Japan, 2006.

26. **Daskalakis V**, Frudakis G, Varotsis C **DFT Study of endoperoxides and their intermediates in Fe(II) cleavage of the endoperoxy bridge**, 19th National Conference in Chemistry, Heraklion, Crete, Greece, 2002.

Detailed list of publication abstracts and 5-year journal impact factors (IF).

**Corresponding Author*

01. V. Daskalakis V* *Protein-Protein Interactions within Photosystem II under Photo-protection: The Synergy between CP29 Minor Antenna, Subunit S (PsbS) and Zeaxanthin at all-atom resolution (2018) Phys. Chem. Chem. Phys.* 20, 11843 – 11855.

Impact Factor: 4.216

The assembly and disassembly of protein complexes within cells are crucial life-sustaining processes. In photosystem II (PSII) of higher plants, there is a delicate yet obscure balance between light harvesting and photo-protection under fluctuating light conditions, that involves protein-protein complexes. Recent breakthroughs in molecular dynamics (MD) simulations are combined with new approaches herein to provide structural and energetic insight into such a complex between the CP29 minor antenna and the PSII subunit S (PsbS). The microscopic model involves extensive sampling of bound and dissociated states at atomic resolution in the presence of photo-protective zeaxanthin (Zea), and reveals well defined protein-protein cross-sections. The complex is placed within PSII, and macroscopic connections are emerging (PsbS-CP29-CP24-CP47) along the energy transfer pathways from the antenna to the PSII core. These connections explain macroscopic observations in the literature, while the previously obscured atomic scale details are now revealed. The implications of these findings are discussed in the context of the Non-Photochemical Quenching (NPQ) of chlorophyll fluorescence, the down-regulatory mechanism of photosynthesis, that enables the protection of PSII against excess excitation load. Zea is found at the PsbS-CP29 cross-section and a pH-dependent equilibrium between PsbS dimer/monomers and the PsbS-CP29 dissociation/association is identified as the target for engineering tolerant plants with increased crop and biomass yields. Finally, the new MD based approaches can be used to probe protein-protein interactions in general, and the PSII structure provided can initiate large scale molecular simulations of the photosynthetic apparatus, under NPQ conditions.

02. Daskalakis V*, and Papadatos S *The Photosystem II Subunit S under Stress (2017) Biophys. J. (Cell)* 113 (11), 2364-2372.

Impact Factor: 3.743

Non-Photochemical Quenching (NPQ) is the protective mechanism against overexcitation of Photosystem II (PSII) triggered by excess ΔpH in photosynthetic membranes. The Light Harvesting Complexes (LHCs), the de-epoxidation of Violaxanthin to Zeaxanthin, and the PSII subunit S (PsbS) work in synergy for an optimized multilevel response. Understanding the fine details of this synergy has proven challenging to scientific research. Here we employ large-scale all-atom molecular simulations and beyond experimental insight, we proceed a step further in identifying the PsbS dynamics that could possibly be associated with this synergy. For the first time, we probe distinct behavior of the PsbS (a) under ΔpH that probes the details of the potential dimer to monomer transition, and (b) in a Violaxanthin/ Zeaxanthin-rich membrane, at an all atom resolution. We propose that the lumen exposed residues Threonine-162 and Glutamic-173 form stabilizing hydrogen bonds between the PsbS monomers only at high lumen pH, whereas at low pH (excess ΔpH) this interaction is lost and leads to

higher flexibility of the protein and potentially to the dimer to monomer transition. Lastly, we discuss how conformational changes under the presence of ΔpH / Zeaxanthin are related to the PsbS role in the current NPQ model in the literature. For the latter, we probe a PsbS-monomeric LHCII association. The association is proposed to potentially alter the monomeric LHCII sensitivity to ΔpH by changing the pKa values of interacting LHCII residues. This serves as an example where protonation-ligation events enhance protein-protein interactions fundamental to many life processes.

03. Papadatos S, Charalambous C A, and **Daskalakis V*** *A pathway for protective quenching in antenna proteins of Photosystem II (2017) Scientific Reports 7, 2523.*
doi: 10.1038/s41598-017-02892-w.

Impact Factor: 4.847

Photosynthesis is common in nature, converting sunlight energy into proton motive force and reducing power. The increased spectral range absorption of light exerted by pigments (i.e. chlorophylls, Chls) within Light Harvesting Complexes (LHCs) proves an important advantage under low light conditions. However, in the exposure to excess light, oxidative damages and ultimately cell death can occur. A down-regulatory mechanism, thus, has been evolved (non-photochemical quenching, NPQ). The mechanistic details of its major component (qE) are missing at the atomic scale. The research herein, initiates on solid evidence from the current NPQ state of the art, and reveals a detailed atomistic view by large scale Molecular Dynamics, Metadynamics and *ab initio* Simulations. The results demonstrate a complete picture of an elaborate common molecular design. All probed antenna proteins (major LHCII from spinach-pea, CP29 from spinach) show striking plasticity in helix-D, under NPQ conditions. This induces changes in Qy bands in excitation and absorption spectra of the nearby pigment pair (Chl613-614) that could emerge as a new quenching site. Zeaxanthin enhances this plasticity (and possibly the quenching) even at milder NPQ conditions.

04. Ioannou A, **Daskalakis V**, and Varotsis C* *Detection of Maillard reaction products by a coupled HPLC-Fraction collector technique and FTIR characterization of Cu(II)-complexation with the isolated species (2017) J. Mol. Struct. 1141, 634–642.*

Impact Factor: 1.561

The isolation of reaction products of asparagine with reducing sugars at alkaline pH and high temperature has been probed by a combination of high performance liquid chromatography (HPLC) coupled with a Fraction Collector. The UV-VIS and FTIR spectra of the isolated Maillard reaction products showed structure-sensitive changes as depicted by deamination events and formation of asparagine-saccharide conjugates. The initial reaction species of the Asn-Glu reaction were also characterized by Density Functional Theory (DFT) methods. Evidence for Cu (II) metal ion complexation with the Maillard reaction products is supported by UV-VIS and FTIR spectroscopy.

05. Ioannou A, Lambrou A, **Daskalakis V*** and Pinakoulaki E* *Coupling of helix E-F motion with the O-nitrito and 2-nitrovinyl coordination in myoglobin (2017) Biophys. Chem. 221, 10-16.*

Impact Factor: 2.121

Myoglobin is known to react slowly with nitrite to form the green pigment by NO_2^- coordination to the heme Fe in the O-binding nitrito (O1-N=O2) mode and to the heme 2-vinyl position forming a reversible low-to high spin species. Nitrite is a powerful oxidizing agent and a biological reservoir for NO that has been implicated in a variety of aerobic biological sys-

tems. Accordingly, it is important to elucidate the nature and variety of NO_2^- reaction mechanisms with Mb. We have performed principal component analysis (PCA, or essential dynamics) on Molecular Dynamics trajectories of all Mb- NO_2^- coordination states to resolve the most important motions in the protein at 298K. We show that the coordination or removal of NO_2^- to/from the heme iron is associated mainly with a motion of helix E and the coordination of NO_2^- to the 2-vinyl is associated with a motion of helix F and a correlated motion of helices E-F. This latter correlated motion can be attributed to the interaction of Val68 and Ile107 with the 2-nitrovinyl moiety. The resonance Raman results show that coordination of NO_2^- to the 2-vinyl is increased at pH 6.0 demonstrating that the amide protons in the F helix are not protected from access of solvent water and the helix F motion allows solvent access to the 2-vinyl group, without affecting the coordination to the heme Fe.

06. Ioannou A, Lambrou A, **Daskalakis V** and Pinakoulaki E* *Nitrite coordination in myoglobin (2017) J. Inorg. Biochemistry (JIB), 166, 49-54.*

Impact Factor: 3.207

The coordination of nitrite in myoglobin (Mb) has been characterized by resonance Raman spectroscopy and the frequencies of the nitrite bound to the heme Fe as well to the 2-vinyl have been computed by Density Functional Theory (DFT) calculations. The DFT Natural Bond Orbital (NBO) analysis and the extensive isotope-labeling in the resonance Raman experiments indicate that NO_2^- ($\text{O}_1\text{-N=O}_2$) is bound to the heme Fe via O_1 . Based on the vibrational characterization of the reversible transition between low and high spin Fe-O-N=O/2-nitrovinyl species, we suggest that the key step that triggers the spin-change is the increase of the proximal Fe-NHis93 bond length. The frequencies of the O and N sensitive bands of the Fe-O-N=O/2-nitrovinyl species remained largely unchanged in the low-to high-spin transition. Therefore the “greening” process in the reaction of ferric Mb with NO_2^- proceeds through the Fe-O-N=O/2-nitrovinyl species, which can exist in either the high or low-spin state.

07. Ioannidis NE, Papadatos S, and **Daskalakis V*** *Energizing the Light Harvesting Antenna: Insight from CP29 (2016) BBA – Bioenergetics, 1857 (10), 1643-1650*

Impact Factor: 5.468

How do plants cope with excess light energy? Crop health and stress tolerance are governed by molecular photoprotective mechanisms. Protective exciton quenching in plants is activated by membrane energization, via unclear conformational changes in proteins called antennas. Here we show that pH and salt gradients stimulate the response of such an antenna under low and high energization by all-atom Molecular Dynamics Simulations. Novel insight establishes that helix-5 (H5) conformation in CP29 from spinach is regulated by chemiosmotic factors. This is selectively correlated with the chl-614 macrocycle deformation and interactions with nearby pigments, that could suggest a role in plant photoprotection. Adding to the significance of our findings, H5 domain is conserved among five antennas (LHCB1–5). These results suggest that light harvesting complexes of Photosystem II, one of the most abundant proteins on earth, can sense chemiosmotic gradients via their H5 domains in an upgraded role from a solar detector to also a chemiosmotic sensor.

08. Salameh A, Vorka, F, and **Daskalakis V*** *Correlation Between Surface Tension and the Bulk Dynamics in Salty Atmospheric Aquatic Droplets (2016) J. Phys. Chem. C, 120 (21), 11508-11518*

Impact Factor: 4.693

The properties of the liquid– gas interface in the presence of surface-active organic matter, and the bulk response to changes of the resulting surface tension are key factors in assessing cloud microphysics or condensation/ evaporation dynamics for atmospheric particles and biological systems alike. A fundamental question rises on how we can induce changes in the bulk of an aquatic droplet by varying its surface-activity. The challenging aspect of answering such a question rises from the fact that water exerts complexity in its interactions giving rise to many anomalous properties. Here, we employ molecular dynamics simulations on salty aquatic droplets, associated with new particle formation in the atmosphere. We present a correlation between surface tension and a bulk property like the static dielectric constant, which is closely related to the water activity in Köhler theory of droplet formation and growth. We thus correlate surface to bulk properties. The particles we probe consist of water, salt (ammonium bisulfate), and surface-active organic matter. A particularly useful result of our study is that surface-active organic matter on the surface of a salty droplet can alter the solvent properties of water in terms of water orientation/water activity measured by the static dielectric constant in the bulk.

09. Daskalakis V, Ohta T, Kitagawa T, Varotsis C* Structure and properties of the catalytic site of nitric oxide reductase at ambient temperature (2015) *Biochim. Biophys. Acta – Bioenergetics*, 1847(10), 1240–1244.

Impact Factor: 5.468

Nitric oxide reductase (Nor) is the third of the four enzymes of bacterial denitrification responsible for the catalytic formation of laughing gas (N_2O). Here we report the detection of the hyponitrite ($HO-N=N-O^-$) species ($\nu_{N-N} = 1332\text{ cm}^{-1}$) in the heme b_3 $Fe-Fe_B$ dinuclear center of Nor from *Paracoccus denitrificans*. We have also applied density functional theory (DFT) to characterize the bimetallic-bridging hyponitrite species in the reduction of NO to N_2O by Nor and compare the present results with those recently reported for the N–N bond formation in the ba_3 and caa_3 oxidoreductases from *Thermus thermophiles*.

10. Daskalakis V*, Charalambous F, Demetriou DC, Georgiou AG *Surface-Active Organic Matter induces salt morphology transitions during new atmospheric particle formation and growth* (2015) *RSC Adv.*, 5, 63240–63251.

Impact Factor: 3.30

The creation of new atmospheric particles via nucleation is an important source of the particles from which Cloud Condensation Nuclei (CCN) are formed. The new particle formation (NPF) process can significantly alter the atmospheric aerosol size distribution and thus CCN activation. CCN are associated with boundary layer evolution, cloud formation, and cloud properties like albedo, or the lifetime. Water vapor condenses upon atmospheric particulates that grow in size to form cloud droplets. Despite its importance, NPF is poorly understood at the atomic level and the ns time scale especially when organic matter (OM) effects are included. Here we employ molecular dynamics simulations on ammonium chloride wet aerosol models. Salt within the aerosol is found to transition between different morphologies – brine and crystalline – depending on the presence of OM on the surface of the occurring particle. Particle number, size and growth dynamics are associated with this variant salt morphology. Our findings elucidate the dynamics of NPF and particle growth in the presence of OM.

11. Daskalakis V*, Charalambous F, Panagiotou F, Nearchou I *Effects of Surface Activity on Carbon Dioxide Nucleation in Atmospheric Wet Aerosols: A Molecular Dynamics Study* (2014) *Phys. Chem. Chem. Phys.*, 16: 23723-23734.

Impact Factor: 4.216

Organic matter (OM) uptake in cloud droplets produces water-soluble secondary organic aerosols (SOA) via aqueous chemistry. These play a significant role in aerosol properties. We report the effects of OM uptake in wet aerosols, in terms of the dissolved-to-gas carbon dioxide nucleation using molecular dynamics (MD) simulations. Carbon dioxide has been implicated in the natural rainwater as well as seawater acidity. Variability of the cloud and raindrop pH is assumed in space and time, as regional emissions, local human activities and geophysical characteristics differ. Rain scavenging of inorganic SO_x, NO_x and NH₃ plays a major role in rain acidity in terms of acid–base activity, however carbon dioxide solubility also remains a key parameter. Based on the MD simulations we propose that the presence of surface-active OM promotes the dissolved-to-gas carbon dioxide nucleation in wet aerosols, even at low temperatures, strongly decreasing carbon dioxide solubility. A discussion is made on the role of OM in controlling the pH of a cloud or raindrop, as a consequence, without involving OM ionization equilibrium. The results are compared with experimental and computational studies in the literature.

12. Daskalakis V*, Hadjicharalambous M *Hexagonal Ice Stability and Growth in the presence of Glyoxal and Secondary Organic Aerosols (2014) Phys. Chem. Chem. Phys.* 16(33): 17799-17810.

2014/2015 Impact Factor: 4.216

The presence of ice dominates the microphysics of formation of high altitude cirrus and polar stratospheric clouds, as well as the maturity of thunderstorms. We report on the hexagonal (1_h) ice stability and growth in binary as well as multi-compound aerosols in atmospherically relevant conformations. The ubiquitous atmospheric trace gas glyoxal along with secondary organic aerosol (SOA) also in the presence of CO₂ interacts with large ice 1_h crystals of 1300–2000 water molecules. The crystals are subjected to phase transitions under superheating and supercooling conditions by Molecular Dynamics (MD) simulations. Density Functional Theory (DFT) based geometry optimization and vibrational frequency analysis are also employed for a smaller ice 1_h cell of 12 water molecules. The interaction of the latter with each organic molecule reveals the extent of the mechanical stress exerted on the ordered ice structure. Full hydration of glyoxal promotes ice 1_h stability and growth in wet aerosols, while partial hydration or full oxidation exerts a destabilizing effect on the ice 1_h lattice. This behavior is associated with the ability of each organic phase to match the order of the ice 1_h crystal. We propose that aqueous chemistry in wet aerosols may also have a strong effect on the microphysics of cloud formation.

13. Pinakoulaki E, Daskalakis V, Ohta T, Richter O M, Budiman K, Kitagawa T, Ludwig B, Varotsis C* *The protein effect in the structure of two ferryl-oxo intermediates at the same oxidation level in the heme-copper binuclear center of cytochrome c oxidase. (2013) J. Biol. Chem. (JBC)* 288(28): 20261-20266.

Impact Factor: 4.441

Identification of the intermediates and determination of their structures in the reduction of dioxygen to water by cytochrome *c* oxidase (CcO) are particularly important to understanding both O₂ activation and proton pumping by the enzyme. In this work, we report the products of the rapid reaction of O₂ with the mixed valence form (Cu_A²⁺, heme *a*³⁺, heme *a*₃²⁺-Cu_B¹⁺) of the enzyme. The resonance Raman results show the formation of two ferryl-oxo species with characteristic Fe(IV)=O stretching modes at 790 and 804 cm⁻¹ at the peroxy oxidation level (PM). Density functional theory calculations show that the protein environment of the proximal H-bonded His-411 determines the strength of the distal Fe(IV)=O bond. In contrast to previous proposals, the PM intermediate is also formed in the reaction of Y167F with O₂.

These results suggest that in the fully reduced enzyme, the proton pumping $\nu_{\text{Fe(IV)=O}}=804\text{ cm}^{-1}$ to $\nu_{\text{Fe(IV)=O}}=790\text{ cm}^{-1}$ transition (P→F, where P is peroxy and F is ferryl) is triggered not only by electron transfer from heme a to heme a_3 but also by the formation of the H-bonded form of the His-411-Fe(IV)=O conformer in the proximal site of heme a_3 . The implications of these results with respect to the role of an O=Fe(IV)-His-411-H-bonded form to the ring A propionate of heme a_3 -Asp-399-H₂O site and, thus, to the exit/output proton channel (H₂O) pool during the proton pumping P→F transition are discussed. We propose that the environment proximal to the heme a_3 controls the spectroscopic properties of the ferryl intermediates in cytochrome oxidases.

14. Porrini M, Daskalakis V, Farantos SC* *Exploring the Topography of Free Energy Surfaces and Kinetics of Cytochrome c Oxidases interacting with small ligands (2012) RSC Adv.* 2: 5828 - 5836.

Impact Factor: 3.30

Free energy landscape explorations have been performed for Cytochrome *c* Oxidases, aa_3 from *Paracoccus denitrificans* and ba_3 from *Thermus thermophilus*, interacting with small gas molecules (CO, NO, O₂), as well as Xe. The calculations were carried out with thermodynamic perturbation theory, the validity of which has been examined by previous molecular dynamics calculations. This approach allows us to bypass the immense computational time required in such problems. The free energy surfaces are constructed as functions of the three Cartesian coordinates of the center of mass of the ligand and averaging over the orientation angles of the molecule. Hydrophilic/hydrophobic cavities and channels around the distal heme- a_3 pocket were detected and the corresponding free energy minima and barriers were estimated. These free energy extrema permit us to extract kinetic parameters and to discuss the biochemical functions of the enzymes in conjunction with experimental results. The conserved cavities found in the two enzymes as well as in previous results of myoglobin demonstrate that topographical characteristics in the distal region of the active sites of the heme oxidase proteins are structurally stable.

15. Kampanarakis A, Farantos SC*, Daskalakis V and Varotsis C *Non-Linear Vibrational Modes in Biomolecules: a periodic orbits description. (2012) Chem. Phys. Elsevier*, 399: 258–263.

Impact Factor: 1.747

The vibrational harmonic normal modes of a molecule, which are valid at energies close to an equilibrium point (a minimum, maximum or saddle of the potential energy surface), are extended by periodic orbits to high energies where anharmonicity and coupling of the degrees of freedom are significant. In this way the assignment of the spectra, and thus the extraction of dynamics in highly excited molecules, can be obtained. New vibrational modes emanating from bifurcations of periodic orbits and long living localized trajectories signal the birth and localization of new quantum states. In this article we review and further study non-linear vibrational modes for model biomolecules such as alanine dipeptide and the active site in the oxoferryl oxidation state of the enzyme cytochrome *c* oxidase. We locate periodic orbits which exhibit high anharmonicity and lead to center saddle bifurcations. These modes are associated to an isomerization process in alanine dipeptide and to frequency shifts in the oxoferryl observed by modifying the Coulomb field around the Imidazole-Fe^{IV}=O species.

16. Pinakoulaki E, Daskalakis V and Varotsis C* (All authors have contributed equally to this work) *The origin of the Fe^{IV}=O intermediates in cytochrome aa_3 oxidase. (2012) BBA Bioenergetics*, 1817: 552-557.

Impact Factor: 5.468

The dioxygen reduction mechanism in cytochrome oxidases relies on proton control of the electron transfer events that drive the process. Proton delivery and proton channels in the protein that are relevant to substrate reduction and proton pumping are considered, and the current status of this area is summarized. We propose a mechanism in which the coupling of the oxygen reduction chemistry to proton translocation (P→F transition) is related to the properties of two groups of highly conserved residues, namely, His411/G386-T389 and the heme a_3 -propionate-A-D399-H403 chain. This article is part of a Special Issue entitled: Respiratory Oxidases.

17. Daskalakis V, Farantos SC, Guallar V, Varotsis C* *Regulation of Electron and Proton Transfer by the Protein Matrix of Cytochrome c Oxidase. (2011) J. Phys. Chem. B, 115(13): 3648-3655.*

Impact Factor: 3.33

Cytochrome *c* oxidase (CcO) catalyzes the four-electron reduction of molecular oxygen to water and couples this reduction to the pumping of four protons through the protein matrix. Water molecules inside the protein are involved in the proton pumping activity as proton carriers. A highly conserved water molecule, among different CcO enzymes, lies between the heme a_3 propionates. Here, we show, by quantum mechanical/ molecular mechanical (QM/MM) simulations, that this conserved water molecule can transfer its proton to propionate-A. His403 residue coordinates to the Mg site near the so-called water pool. By both QM/MM and molecular dynamics calculations, we demonstrate that the also conserved His403 residue, adjacent to the heme a_3 propionate-A, plays a role of a valve controlling the protonation state of the propionate-A/Asp399 pair. This, in turn, controls the oxidation state of the heme a_3 iron, linking in this way, the D-proton pathway to the water pool.

18. Daskalakis V*, Farantos SC, Guallar V, Varotsis C *Vibrational Resonances and Cu_B displacement controlled by proton motion in Cytochrome c Oxidase, (2010) J. Phys. Chem. B, 114(2): 1136-1143.*

Impact Factor: 3.33

Cytochrome *c* oxidase (CcO), found in the inner mitochondrial membranes or in many bacteria, catalyzes the four-electron reduction of molecular oxygen to water. Four protons are pumped across the inner mitochondrial membrane through CcO. In this study, quantum mechanics/molecular mechanics and molecular dynamics calculations are used to probe the spectroscopic characteristics of the ferryl intermediates in the aa_3 CcO/O₂ reaction. These highly elaborate calculations, supported by several calculations on smaller model systems, demonstrate the sensitivity of vibrational frequencies on the Coulombic field of heme a_3 and their dependence on the distance of the adjacent Cu_B to the heme a_3 Fe atom. This distance seems to be associated with the protonation state of the heme a_3 propionate A, and we propose that it plays a crucial role on the mechanism of action of CcO. In detail, we link proton pumping activity in CcO enzyme (a) to a multiple (1:1:2) resonance among the frequencies of Fe^{IV}=O bond stretching, the breathing mode of Histidine 411, and a bending mode of the His411-Fe^{IV}=O species (aa_3 from *Paracoccus denitrificans* numbering) and (b) to Cu_B displacement by electrostatic interactions toward the heme a_3 iron. We find that the vibrations of the His411-Fe^{IV}=O unit become highly coupled depending on the protonation state of the heme a_3 ring A propionate/Asp399 pair, and we propose a mechanism for the resonance Raman enhancement of the bending mode δ (His411-Fe^{IV}=O). Calculations on model systems demonstrate that the position of Cu_B in relation to heme a_3 iron-oxo plays a crucial role in regulating that resonance. We also discuss the origin of the coupling between bending, δ (His411-

Fe^{IV}=O) and $\nu(\text{Fe}=\text{O})$ stretching modes, and the role played by such vibrational coupling interactions or Cu_B position in controlling functional properties of the enzyme, including electron/proton coupling as well as experimental spectra.

19. Daskalakis V, Varotsis C* *Binding and Docking Interactions of NO, CO and O₂ in Heme Proteins as Probed by Density Functional Theory, Review for the Special Issue on "Application of Density Functional Theory in Chemical Reactions"*. (2009) *Int. J. Mol. Sci.*, 10: 4137-4156.

Impact Factor: 3.482

Dynamics and reactivity in heme proteins include direct and indirect interactions of the ligands/substrates like CO, NO and O₂ with the environment. Direct electrostatic interactions result from amino acid side chains in the inner cavities and/or metal coordination in the active site, whereas indirect interactions result by ligands in the same coordination sphere. Interactions play a crucial role in stabilizing transition states in catalysis or altering ligation chemistry. We have probed, by Density Functional Theory (DFT), the perturbation degree in the stretching vibrational frequencies of CO, NO and O₂ molecules in the presence of electrostatic interactions or hydrogen bonds, under conditions simulating the inner cavities. Moreover, we have studied the vibrational characteristics of the heme bound form of the CO and NO ligands by altering the chemistry of the proximal to the heme ligand. CO, NO and O₂ molecules are highly polarizable exerting vibrational shifts up to 80, 200 and 120 cm⁻¹, respectively, compared to the non-interacting ligand. The importance of Density Functional Theory (DFT) methodology in the investigation of the heme-ligand-protein interactions is also addressed.

20. Porrini M*, Daskalakis V, Farantos SC, Varotsis C *Heme Cavity Dynamics of Photodissociated CO from ba₃-Cytochrome c Oxidase: The Role of Ring-D Propionate*, (2009) *J. Phys. Chem. B*, 113(35): 12129-12135.

Impact Factor: 3.33

Intracavity molecular dynamics studies of photodissociated carbon monoxide from ba₃-cytochrome c oxidase have been performed by sampling the phase space with several hundreds of trajectories each integrated up to 100 ps time interval. It is shown that the cis conformation of protonated ring-D propionate of heme a₃ and its trans conformation for the deprotonated species control the CO location by creating two distinct equilibrium states for CO confined in a cavity internal to the distal heme pocket. Thus, these cis (closed gate) and trans (open gate) conformations of heme a₃ propionate D play the role of a switch, opening or closing a gate for confining CO in a cavity internal to the heme pocket or releasing it to a bigger outer cavity. The geometry of the inner cavity and the validity of the potential function employed are further investigated by Density Functional Theory calculations for the active site, potential of mean force curves along the copper-CO bond, as well as with Quantum Mechanics/Molecular Mechanics calculations. In the light of the present study, trajectory scenarios for the dissociation of CO previously suggested from time-resolved infrared spectroscopy are re-examined.

21. Daskalakis V, Farantos SC*, Varotsis C *Assigning vibrational spectra of ferryl-oxo intermediates of cytochrome c oxidase by periodic orbits and Molecular Dynamics*, (2008) *J. Am. Chem. Soc* 130(37): 12385-12393.

Impact Factor: 12.226

Complexity is inherent in biological molecules not only because of the large number of atoms but also because of their nonlinear interactions responsible for chaotic behaviors, localized motions, and bifurcation phenomena. Thus, versatile spectroscopic techniques have been invented to achieve temporal and spacial resolution to minimize the uncertainties in assigning the spectra of complex molecules. Can we associate spectral lines to specific chemical bonds or species in a large molecule? Can energy stay localized in a bond for a substantial period of time to leave its spectroscopic signature? These longstanding problems are investigated by studying the resonance Raman spectra of ferryl-oxo intermediates of cytochrome c oxidase. The difference spectra of isotopically substituted ferryl oxygen (^{16}O minus ^{18}O) in the cytochrome c oxidase recorded in several laboratories show one or two prominent positive peaks which have not been completely elucidated yet. By applying the hierarchical methods of nonlinear mechanics, and particularly the study of periodic orbits in the active site of the enzyme, in conjunction with molecular dynamics calculations of larger systems which include the embraced active site by the protein and selected protonated/deprotonated conformations of amino acids, we translate the spectral lines to molecular motions. It is demonstrated that for the active site stable periodic orbits exist for a substantial energy range. Families of periodic orbits which are associated with the vibrations of $\text{Fe}^{\text{IV}}=\text{O}$ bond mark the regions of phase space where nearby trajectories remain localized, as well as assign the spectral bands of the active site in the protein matrix. We demonstrate that proton movement adjacent to active site, which occurs during the $\text{P} \rightarrow \text{F}$ transition, can lead to significant perturbations of the $\text{Fe}^{\text{IV}}=\text{O}$ isotopic difference vibrational spectra in cytochrome c oxidase, without a change in oxidation state of the metal sites. This finding links spectroscopic characteristics to protonation events occurring during enzymatic turnover.

22. Daskalakis V, Pinakoulaki E, Stavarakis S, Varotsis C* *Probing the environment of Cu_B in heme-copper oxidases. (2007) J. Phys. Chem. B 111: 10502-10509.*

Impact Factor: 3.30

Time-resolved step-scan FTIR (TRS²-FTIR) and density functional theory have been applied to probe the structural dynamics of Cu_B in heme-copper oxidases at room temperature. The TRS²-FTIR data of *cbb₃* from *Pseudomonas stutzeri* indicate a small variation in the frequency of the transient CO bound to Cu_B in the pH/pD 7-9 range. This observation in conjunction with density functional theory calculations, in which significant frequency shifts of the $\nu(\text{CO})$ are observed upon deprotonation and/or detachment of the Cu_B ligands, demonstrates that the properties of the Cu_B ligands including the cross linked tyrosine, in contrast to previous reports, remain unchanged in the pH 7-9 range. We attribute the small variations in the $\nu(\text{CO})$ of Cu_B to protein conformational changes in the vicinity of Cu_B . Consequently, the split of the heme Fe-CO vibrations (α -, β -, and γ -forms) is not due to changes in the ligation and/or protonation states of the Cu_B ligands or to the presence of one or more ionizable groups, as previously suggested, but the result of global protein conformational changes in the vicinity of Cu_B which, in turn, affect the position of Cu_B with respect to the heme Fe.

23. Pinakoulaki E, Yoshimura H, Daskalakis V, Yoshioka S, Aono S, Varotsis C* *Two ligand binding sites in the O_2 -sensing signal transducer HemAT: Implications for ligand Recognition/ Discrimination and signaling. (2006) Proc. Natl. Acad. Sci. USA 103: 14796-14801.*

Impact Factor: 10.4

We have identified a ligand (CO) accommodation cavity in the signal transducer sensor protein HemAT (heme-based aerotactic transducer) that allows us to gain single-molecule insights into the mechanism of gas sensor proteins. Specific mutations that are distal and prox-

imal to the heme were designed to perturb the electrostatic field near the ligand that is bound to the heme and near the accommodated ligand in the cavity. We report the detection of a second site in heme proteins in which the exogenous ligand is accommodated in an internal cavity. The conformational gate that directs the ligand-migration pathway from the distal to the proximal site of the heme, where the ligand is trapped, has been identified. The data provide evidence that the heme pocket is the specific ligand trap and suggest that the regulatory mechanism may be tackled starting from more than one position in the protein. Based on the results, we propose a dynamic coupling between the two distinct binding sites as the underlying allosteric mechanism for gas recognition_discrimination that triggers a conformational switch for signaling by the oxygen sensor protein HemAT.

24. Pinakoulaki E, Koutsoupakis C, Stavrakis S, Marialena A, Papadopoulos G, **Daskalakis V**, Varotsis C* *Structural dynamics of Heme-copper oxidases and Nitric oxide reductases: Time-Resolved step-scan FTIR and Time-resolved Resonance Raman studies.* (2005) *J. Raman Spec.* 36: 337-349.

Impact Factor: 2.647

Of the spectroscopic methods available for the characterization of the dynamics of heme protein active sites, time resolved resonance Raman spectroscopy (TR³) is a powerful technique because excitation within the heme $\pi \rightarrow \pi^*$ electronic absorption transitions selectively enhances vibrational modes of the heme and bound-proximal/distal ligands without the interference from the modes associated with the protein matrix. On the other hand, time resolved step-scan (TRS²) Fourier transform infrared (FTIR) spectroscopy has the sensitivity and resolution to detect, in addition to ligands bound to metal centers and the kinetics of ligand photodissociation, transient changes at the level of individual amino acids during protein action. This review outlines the application of both TR³ and TRS²-FTIR to heme-copper oxidases and nitric oxide reductases.

25. Koutsoupakis C, Pinakoulaki E, Stavrakis S, Daskalakis V, Varotsis C* Time-resolved step-scan Fourier transform infrared investigation of heme-copper oxidases: implications for O₂ input and H₂O/H⁺ output channels. (2004) *Bioch. Biophys. Acta* 1655: 347-352.

Impact factor: 5.468

We have applied FTIR and time-resolved step-scan Fourier transform infrared (TRS²-FTIR) spectroscopy to investigate the dynamics of the heme-Cu_B binuclear center and the protein dynamics of mammalian *aa₃*, *Pseudomonas stutzeri cbb₃*, and *caa₃* and *ba₃* from *Thermus thermophilus* cytochrome oxidases. The implications of these results with respect to (1) the molecular motions that are general to the photodynamics of the binuclear center in heme copper oxidases, and (2) the proton pathways located in the ring A propionate of heme *a₃*-Asp372-H₂O site that is conserved among all structurally known oxidases are discussed.