ALLEGATO B

UNIVERSITÀ DEGLI STUDI DI MILANO
selezione pubblica per n.1 posto di Ricercatore a tempo determinato ai sensi dell'art.24, comma 3, lettera b) della Legge 240/2010 per il settore concorsuale 05/E1 - BIOCHIMICA GENERALE , settore scientifico-disciplinare BIO/10 - BIOCHIMICA presso il Dipartimento di Biotecnologie Mediche e Medicina Translazionale, (avviso bando pubblicato sulla G.U. n. 53 del 05-07-19) Codice concorso 4119

Stefano Vavassori

CURRICULUM VITAE

INFORMAZIONI PERSONALI (NON INSERIRE INDIRIZZO PRIVATO E TELEFONO FISSO O CELLULARE)

COGNOME	**VAVASSORI**
NOME	**STEFANO**
DATA DI NASCITA	12,12, 1979

ISTRUZIONE E FORMAZIONE:

Project Management Professional (PMP)® **03.2018**

Project Management Institute (USA)

Master in Management di Pharma/Biotech/Medtech **07.2015**

Ecole poyltechnique fédérale de Lausanne (CH)
Specializzazioni: *Drug discovery management, Clinical Trial Management & Regulatory Affairs, cGMPs, Marketing in Life science and Project Management.*

Ph.D. in Biologia Cellulare e Molecolare **07.2010**

Università Vita-Salute San Raffaele (IT) e London Open University (UK)
Titolo della tesi*: Structural and functional characterization of human ERp44: a closer look at a member of PDI family regulating protein quality control in the early secretory pathway.*

Laurea in Biotecnologie **04.2004**

Università degli Studi di Milano.
Titolo della tesi *: Interazione fra il Chaperone BiP e proteine di riserva, naturali e chimeriche, espresse in piante transgeniche di Tabacco.*
Votazione: 110/110 e lode

ESPERIENZE DI RICERCA:

R&D Project Manager **04.2016-present**

Dipartimento R&D, Philip Morris International (CH).

Senior Research Scientist **09.2011-03.2016**

Dipartimento di Biochimica, Università di Losanna (CH).
Laboratorio Professor Andreas Mayer. Progetto*: Characterization of the molecular mechanisms of membrane fusion.*

Visiting Scientist **09.2010-12.2010**

Dipartimento di Biochemica, Kyushu University (JP).
Laboratorio Professor Kenji Inaba. Progetto: *Antibody polymerization (IgM) in vitro.*

Ricercatore Post-Dottorato **07.2010-08.2011**

DiBiT, San Raffaele Scientific Institute (IT)
Laboratorio Professor Roberto Sitia. Progetto: *Regulation of antibody production in human cell lines.*

Studente di Dottorato **01.2006-07.2010**

DiBiT, San Raffaele Scientific Institute (IT)
Laboratorio Professor Roberto Sitia e Dottor Massimo Degano. Progetto: *Molecular Chaperones that control protein assembly in human cell lines.*

Ricercatore Junior **09.2004-12.2005**

Dulbecco Telethon Institute, San Raffaele Scientific Institute (IT)
Laboratorio Dottor Luca Rampoldi. Progetto: *Cellular model of a rare protein aggregation disease (MCKD/FJHN).*

Studente di Tesi **10.2002-06.2004**

Istituto Biologia e Biotecnologie Agrarie, CNR (IT)
Laboratorio Dottor Alessandro Vitale. Progetto: *Production of recombinant pharmaceutical proteins in plant.*

ESPERIENZA DI INSEGNAMENTO:

Junior Lecturer di Biochimica e Proteomica **2011-2016**

Università di Losanna (Svizzera)

Supevisore di studenti univesitari di Medicina e Biotecnologie **2006-2009**

Università Vita-Salute San Raffaele (Italia)

Tutor di studenti univerisitari di Biotecnologie **2003-2004**

Università di Milano (Italia)

CONOSCENZA IT:

- **Layout**

MS Office Excel, Powerpoint, Word, Adobe suit

- **Statistics**

Excel, GraphPad

- **Management**

MS Project, Mindjet manager

- **Biology**

PubMed, Embase, PDB, Mendeley

PREMI E BORSE DI STUDIO:

- Peer-reviewer per *PNAS* e *Int. J. Mol. Sci.*
- Peer-reviewer per *Rustaveli National Science Foundation–Georgia, 2011-2014*
- Società italiana di Biologia Cellulare, ABCD Grant Young Investigators, 2011
- Journal of Cell Science Travelling Fellowship, 2011
- San Raffaele Scientific Institute, Young investigators award, 2006 and 2007
- Borsa di Dottorato Fondazione Cariplo, 2006-2010
- Borsa di studio universitaria, 1998-2004

CAPACITÀ TECNICHE:

Interazione proteina-proteina:

Surface plasmon resonance, ITC, Microscale thermophoresis, GST-pull down, peptide array, IP.

Purificazione e Caratterizzazione di proteine:
Ion-exchange, reversed-phase, size exclusion and affinity chromatography, electrophoresis, immunoblotting.

Imaging:

Fluorescence and confocal microscopy, Label-free non-invasive 3D imaging (Nanolive technology).

Biologia strutturale:

Protein crystallization, DLS, synchrotron activity, spectroscopic techniques.

Biologia cellulare:

Metabolic radio-labelling, organelle gradient, SDS-PAGE, western Blot, immunoprecipitation, ELISA. Bacteria, plant cell, yeast cell and mammalian cell lines culture techniques.

Biologia molecolare:

PCR, molecular cloning, protein expression in bacterial, yeast and mammalian systems. DNA and siRNA transfection, KO and establishing stable cell lines.

Gestione persone e progetti:

Supervisor of Master and PhD students, grant writing & peer-review, people & project & budget management in multinational companies, identify & develop collaboration with stakeholders in R&D companies, startups, academia, hospitals and civil society.

LINGUE:

- Italiano (Lingua Madre)
- Inglese (Fluente)
- Francese (B2)

SINTESI DEI PRINCIPALI RISULTATI OTTENUTI:

Profilo del candidato:

La mia attività di ricerca è principalmente collegata a diversi aspetti inerenti l'assemblaggio, quality control e il targeting & secrezione di proteine. Più recentemente ho iniziato a occuparmi di lisosomi e del ruolo di specifiche proteine nella fusione & fissione e biogenesi di membrane lisosomiali.
Ho lavorato con 3 sistemi modello: piante, uomo e lieviti. Ho lavorato in 5 paesi (Italia, Francia, Giappone, Svizzera e Israele)
Nel periodo aziendale ho affinato le mie soft skills, le mie capacita' di management e acquisendo esperienza e competenze per facilitare i rapporti fra accademia e impresa&start-ups.

Scoperte scientifiche principali durante il periodo aziendale:

- Nuovi metodi di management di progetti crowd-sourcing (#2, vedere la lista di pubblicazioni sotto in "Produzione scientifica").
- Creazione, validazioni di network biologici computazionali come tool per biologia & tossicologia predittiva (#1).

Scoperte scientifiche principali durante il periodo di ricerca da Post-Doc:

- Regolazione del potenziale ossidante del Reticolo Endoplasmatico via Prx4 e Ero1 (#6).
- La fusione omotipica dei lisosomi avviene in modo indipendente dall'acidificazione di tali organelli(#4, #5).
- Nuovo ruolo della V-ATPase nella fusione di membrane lisosomiali (#3).

Scoperte scientifiche principali durante il periodo di ricerca da studente di dottorato:

- Struttura cristallografica di ERp44 (#10).
- Identificazione the ruolo autoinibitorio del dominio C-terminale di ERp44 (#10).
- ERp44-PDI_Ero1 regolano il folding ossidativo delle protein nel reticolo endoplasmatico (#8,#9).
- Ruolo del gradiente di pH nel regolare il controllo qualità delle protein della via secretoria (#7).

Altre scoperte scientifiche:

- Ruolo del chaperone BiP nel regolare il folding e l'assemblamento di proteine (#12, #13).
- Produzione e accumulo di proteine ricombinanti (Zeolina) in piante di tabacco (#12).
- Modello cellulare di una malattia genetica rara (#11).

Short essay about Post-Doctoral project

Membrane fusion is a fundamental event for traffic between different membrane compartments in eukaryotic cells. In the endomembrane system, SNAREs from opposing membranes form four-helix bundles called SNARE complexes that bring the two membranes into close proximity. This finding led to the proposal that SNAREs can induce membrane fusion, probably by mechanical force and a stalk mechanism involving a lipidic fusion pore. Recent reconstitution experiments indicated, however, that SNAREs reconstituted at physiological protein densities convey only very low fusion activity, and that additional factors are necessary for efficient fusion, such as for Rab proteins and their effectors, SM proteins, synaptotagmin etc.

A role in fusion has also been found for V0 sectors which form the membrane-integral parts of V-ATPase. The V-ATPase is a proton pump consisting of a membrane-integral V0 sector and a peripheral V1 sector, which carries the ATPase activity. V0 sector contains six subunits, a-, d-, the proteolipids c-, c'- and c''-, and subunit e. d-subunit is the only soluble V0 sector. The proteolipids are highly conserved small proteins spanning the membrane four times. In membranes, proteolipids form a hexameric cylinder.

The implication of V0 sector in vacuolar membrane fusion was surprising and stirred controversy because the V-ATPase has a well-established role as a proton pump. Over time, however, unbiased genetic screens identified V0 alleles causing strong in vivo defects in the fusion of synaptic vesicles, multivesicular bodies and phagosomes. In vitro studies suggested that V0 interacts with SNAREs, core components of the fusion machinery, and that vacuole fusion requires physical presence of V0 but not its proton pump activity. A recent in vivo study arrived at the opposite conclusion, using vacuole fusion during the slow mating of yeast cells and the steady state morphology of vacuoles in dividing cells. We re-investigated the role of V-ATPase-dependent vacuolar acidification for vacuole structure and fusion in vivo, using live microscopy on the timescale of minutes.

This new approach allows us to emancipate from the classical steady state vacuole morphology, which it is not strictly correlated with fusion activity because vacuole structure depends on an equilibrium of fusion and fission activities. It, instead, allows us to pinpoint how readily the organelles react to acute perturbation of proton pump activity of V-ATPase. Acute pharmacological or physiological inhibition of V-ATPase proton pump activity induced rapid vacuole fusion. In conclusion, our direct microscopic observations of vacuoles in vivo

indicate that the proton pumping activity and/or electrochemical gradient established by the V-ATPase is not needed for vacuole fusion, even counteracts it

In the second part of my work, we investigated the molecular mechanisms of V-ATPase mediated fusion, using isolated vacuole/lysosome from yeast cells. Using a combination of genetic and biochemical techniques, we demonstrate a novel role, proton-pump independent, of the d-subunit of V-ATPase (vma6p in yeast) in membrane fusion in yeast vacuole. Initially, d-subunit is required for the separation of cis-SNARE complexes (SNAREs priming) and for the release of Sec17p/α-SNAP from SNAREs. After accomplished its role, it is promptly displaced from its binding site in the middle of the proteolipid cylinder to allow fusion proceed efficiently.
Furthermore, a panel of V0 sector mutants show defects in the later steps of fusion, in the transition between hemifusion-fusion. Based on these findings it was suggested that a SNARE-dependent conformational change in the V0 sector might promote lipid reorientation and subsequent formation of a fusion pore.

Due to the conservation of V0, SNAREs and the Rab system these insights will be of broad relevance for fusion reactions in the late secretory and endocytic pathways also in higher eukaryotes and may help to better understand the control and catalysis of vesicular traffic, organelle biogenesis and organelle homeostasis.

Short essay about PhD Project

The Endoplasmic Reticulum (ER), the "gateway" of the secretory pathway, is the site of maturation of secretory proteins in eukaryotic cells. Through its oxidizing environment and its specialized folding helpers, the ER provides an optimal milieu for protein folding and assembly. In the ER, several co-translational and post-translational modifications take place that do not occur in the cytosol, such as disulfide-bond formation, N-linked glycosylation and GPI-anchor addition.
Fidelity of protein-based intracellular communication is guaranteed by tight protein quality control mechanisms located at the Early Secretory Compartment (ESC), which restricts forward transport to native proteins. High fidelity of secretion depends on retention in, or retrieval into the ER, where
immature cargoes are given another chance to complete folding and assembly. The calnexin/calreticulin cycle exploits rounds of de- and reglucosylation to determine glycoprotein capture and release. Chaperone BiP binds hydrophobic surfaces on immature cargoes in an ATP-dependent fashion. Likewise exposure of unpaired cysteines is exploited in retaining assembly intermediates that have undergone incomplete disulfide bond formation. How such Thiol-Mediated Retention (TMR) is achieved *via* capture and release cycles have remained obscure. The ERp44 cycle is paramount in the retrieval of orphan subunits of otherwise disulfide-linked oligomers such as IgM or adiponectin, whose recognition depends on free thiols. Little is known about the molecular mechanisms of ERp44-TMR and how it is regulated in living cells. Hence, the overall aim of my work was to investigate the structure-function relationship of ERp44. The availability of high-resolution structure of a protein is mandatory for understanding its structure-function relationship. It is particularly important to assess at the atomic level the role of the individual domains of ERp44 in modulating its

function. Moreover, three-dimensional structure provided a solid framework for the design of protein mutants of ERp44.
Initially, we determined the crystal structure of human ERp44 at a resolution of 2.6 Å (Protein data bank accession number 2R2J). Three thioredoxin domains -**a**, -**b** and -b' are arranged in a clover-like structure. A flexible carboxy-terminal tail turns back to the **b'** and **a** domains, shielding the Substrate Binding Site (SBS) of ERp44 (Cys29 in domain **a** and surrounding hydrophobic pocket).
The structure of ERp44 most likely represents a non-reactive conformation of the protein. Indeed, Cys29 is shielded from the bulk solvent by C-terminal tail and is almost inaccessible for the formation of intermolecular disulfide bonds with client proteins. Based on the obtained structural data and functional studies, a panel of mutants of ERp44 has been characterized in order to understand how C-terminal tail rearrangements expose substrate binding site, thus modulating substrates binding/release in view of its role in TMR.
Moreover, we showed that ERp44 operates in a pH dependent manner, in synchrony with forward (ERGIC53)- and backward (KDEL-receptors) cargo transporters, to optimize secretion efficiency and fidelity. At ER-equivalent neutral pH, the ERp44 C-terminal tail obscures the thiol-active cysteine and surrounding hydrophobic patches. At the cis-Golgi-equivalent, slightly acidic pH, however, the C-tail becomes flexible, unmasking both the active site to allow capture of client proteins and the RDEL motif to allow retrieval by KDEL receptors. Upon retrieval to the ER, the neutral pH ensures release of client proteins.
In conclusion, ERp44 acts as a pH-dependent chaperone whose C-tail movements couple substrate binding with targeting information. The ERp44, ERGIC-53 and KDEL-R triad epitomizes how relatively small pH variations between intracellular compartments may be exploited to regulate key biological functions, such as protein quality control and secretion.

The studies outlined in my PhD thesis delineate a novel QC system, which works downstream the calnexin/calreticulin- and BiP-dependent cycles. Our model predicts that the lower pH in distal ESC favours opening of the ERp44 C-tail. This simultaneously exposes the active site and the RDEL motif, allowing binding to substrates (e.g. Ero1, SUMF1 or IgM subunits) and KDEL receptors, respectively. A lower pH also favours detachment of ERGIC-53substrates (including IgM and SUMF1) that can be then retrieved backwards.
Besides their remarkable biological interest, a better understanding of the mechanisms underlying protein quality control and transport is relevant medically for the growing number of related 'conformational diseases', and biotechnologically, as it can provide knowledge to produce larger quantities of proteins of interest (e.g. SUMF1 for multiple sulfatase deficiency).

PRODUZIONE SCIENTIFICA:

Dati bibliometrici della produzione scientifica:

- *Total papers:* 11 (+2 submitted)
- *Citazioni totali:* 572
- *Citazioni medie per pubblicazione:* 57.2
- *Citazioni medie per anno ("Publish or Perish" Software) :* 35.75
- *Impact factor totale:* 79.1
- *Impact factor medio:* 7.9
- *H-Index:* 10
- *G-Index ("Publish or Perish" Software):* 10

Nota: Gli impact factor delle riviste sono riferiti all'anno di pubblicazione.

Lista completa delle pubblicazioni su giornali peer-reviewed con IF:

1. L. Schilli, M. Gstrein, J. Jurt, **S. Vavassori**, S. Teufel, M.P. Blanc. "How to manage crowdsourcing projects: an example of independent verification of methods and data in an R&D environment". 2018 Submitted to R&D Management

2. **Vavassori S**, Mayer A. "Role of V0 –V-ATPase in membrane fusion". 2018 Submitted to *Embo J.*

3. Yepiskoposyan H, Talikka M, **Vavassori S**, Martin F, Sewer A, Gubian S, Luettich K, Peitsch MC, Hoeng J. "Construction of a Suite of Computable Biological Network Models Focused on Mucociliary Clearance in the Respiratory Tract". Front Genet. 2019 Feb 15;10:87

4. **Vavassori S**, Desfougères Y, Rompf M, Mayer A. "Homotypic vacuole fusion in living yeast cells is impaired by organelle Acidification". Sci Rep. 2016 Jul 1;6:29045.
   Journal Impact factor 2016 : 4.9
   Citations: 12

5. **Vavassori S**, Mayer A. "A new life for an old pump: V-ATPase and neurotransmitter release.", J. Cell Biology. 2014 Apr 14;205(1):7-9.
   Journal Impact factor 2014 : 9.8
   Citations: 13

6. Kakihana T, Araki K, **Vavassori S**, Iemura SI, Cortini M, Fagioli C, Natsume T, Sitia R, Nagata K. "Dynamic regulation of Ero1α and Prx4 localization in the secretory pathway", J. Biol Chem. 2013
   Journal Impact factor 2013 : 4.6
   Citations: 41

7. **Vavassori S**, Cortini M, Masui S, Sannino S, Anelli T, Caserta IR, Fagioli C, Mossuto MF, Fornili A, van Anken E, Degano M, Inaba K, Sitia R. "A pH-Regulated Quality Control Cycle for Surveillance of Secretory Protein Assembly", Molecular Cell 2013, Jun 27;50(6):783-92.
   Journal Impact factor 2013 : 14.5
   Citations: 50

   Highlights and commentary for this article:
   a. Amy Donner "*Retaining clients*" Nature Chemical Biology 2013, 9, 409
   b. LM Hendershot, MJ Feige, J Buchner "*Acidification Activates ERp44-A Molecular Litmus Test for Protein Assembly*", Molecular Cell, 2013, 50, Issue 6, 27 June 2013, Molecular Cell, 2013
   c. Romisch K. - *Article Recommendation by F1000prime,* Evaluated on 23 May 2013

d. Babu M. and Weatheritt R. - *Article Recommendation by F1000prime*, Evaluated on 7 October 2013

8. Masui S, **Vavassori S**, Fagioli C, Sitia R, Inaba K. "Molecular bases of cyclic and specific disulfide interchange between human ERO1alpha protein and protein-disulfide isomerase (PDI)", J. Biol Chem. 2011 May 6;286(18):16261-71.
   Journal Impact factor 2011 : 4.8
   Citations: 54

9. Inaba K, Masui S, Iida H, **Vavassori S**, Sitia R, Suzuki M. "Crystal structures of human Ero1α reveal the mechanisms of regulated and targeted oxidation of PDI", EMBO J. 2010 Oct 6;29(19):3330-43.
   Journal Impact factor 2010 : 10.2
   Citations: 94

10. Wang L, Wang L*, **Vavassori S***, Li S, Ke H, Anelli T, Degano M, Ronzoni R, Sitia R, Sun F, Wang CC.(2008)" Crystal structure of human ERp44 shows a dynamic functional modulation by its carboxy-terminal tail", EMBO Rep. 2008 Jul;9(7):642-7.
    Journal Impact factor 2008 : 7.1
    Citations: 69
    *These authors contributed equally to this work

11. **Vavassori S**, Bernascone I, Di Pentima A, Santambrogio S, Lamorte G, Amoroso A, Scolari F, Ghiggeri GM, Casari G, Polishchuck R, Rampoldi L. (2006)"Defective intracellular trafficking of uromodulin mutant isoforms", Traffic ,Nov;7(11)1567-79
    Journal Impact factor 2006 : 6.6
    Citations: 84

12. Mainieri D, Rossi M, Archinti M, Bellucci M, De Marchis F, **Vavassori S**, Pompa A, Arcioni S, Vitale A.(2004)."Zeolin, a new recombinant storage protein constructed using maize□□-zein and bean phaseolin", Plant Physiology, Nov;136(3):3447
    Journal Impact factor 2004 : 5.88
    Citations: 117

13. Foresti O, Frigerio L, Holkeri H, de Virgilio M, **Vavassori S**, Vitale A. (2003)."A phaseolin domain involved directly in trimer assembly is a determinant for binding by chaperone BiP", Plant Cell 15:2464-2475.
    Journal Impact factor 2003 : 10.7
    Citations: 38

RELAZIONI SU INVITO E A CONGRESSI NAZIONALI E INTERNAZIONALI

- sbv IMPROVER Epigenomics Challenge Symposium (Tel aviv, Israele), 2017
- European Conference on Computational Biology (L'aia, Olanda), 2016
- 3rd NIBR Novartis– EPFL – UNIL science meeting (Basilea, Svizzera), 2015
- Annual meeting Club Exocytose-Endocytose (Evian-les-Bains, Francia), 2015

- Building the cell , French society of cell biology (Paris, Francia), 2014
- Annual meeting Club exo-endo (L'isle-sur-la-sorgue, Francia), 2012
- Italian society of cell biology "ABCD meeting" (Ravenna, Italia), 2011
- "ER and RedOx club meeting" (Munich, Germania), 2011
- CNR-IBBA (Milano, Italia), 2011
- Kyushu University (Fukuoka, Giappone), 2010
- Kyoto-Sangyo University (Kyoto, Giappone), 2010
- Ph.D. student seminar, University of Cambridge (Cambridge, UK), 2009
- Gordon research Conference "Thiol-based redox regulation and signalling" (Lucca, Italia), 2008
- European Plant Endomembrane Meeting, (Neuchatel, Svizzera), 2004

ATTIVITA DIVULGATIVE

Info-OGM **2002-2005**

Scientific content writer per *info-OGM* (chiuso nel 2005), un sito internet fondato dagli student di Biotecnologia agrarie-vegetali dell' Universita' degli studi di Milano. Il sito si occupava di divulgazione scientifica nel campo degli OGM e, più in generale, sulle biotecnologie.

Life science network **2016-present**

Sono confondatore e moderator del sito LinkeIn *Life Science Networking group Switzerland* (~ 800 members). Il principale scopo del sito e quello di connettere professionisti nel campo delle scienze della vita in Svizzera e di fungere da ponte fra l'accademia, industria e non-profits. (https://www.linkedin.com/groups/8488274).

Le dichiarazioni rese nel presente curriculum sono da ritenersi rilasciate ai sensi degli artt. 46 e 47 del DPR n. 445/2000.

Il presente curriculum, non contiene dati sensibili e dati giudiziari di cui all'art. 4, comma 1, lettere d) ed e) del D.Lgs. 30.6.2003 n. 196.

Autorizzo il trattamento dei dati personali contenuti nel mio curriculum vitae in base ex D.

LGS. 196/03".Aut. Min. N.13/I/007145/03.04 del 1 Aprile 2008.

Data 14 Luglio 2019 Luogo Lausanne