

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Fioriti, Luana

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

POSITION TITLE: Group Leader, Head of Laboratory, Istituto di Ricerche Farmacologiche Mario Negri, IRCCS

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

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Milan, Milan (Italy)	B.Sc.	02/2001	Biology
University of Milan, Italy	M.S.	07/2004	Biotechnology
Open University, London, UK	Ph.D.	02/2006	Pharmacology
Columbia University	Postdoc	10/2010	Neuroscience

A. Personal Statement

The main focus of my research has been to understand the cellular and molecular aspects of protein aggregation, in the context of both pathological and functional prions. The ultimate goal of my research is to design novel therapeutic approaches that might be effective in preventing or delaying the onset of neurodegenerative. To this end, since the beginning of my independent position in 2017, I started to investigate the role of post-translational modifications in the modulation of aggregation, toxicity and release of aggregation prone proteins, such as tau, Htt and synuclein. In particular, my laboratory focuses on understanding the contribution of SUMOylation as a regulator of protein aggregation utilizing both *in vitro* and *in vivo* models of Alzheimer's Disease and frontotemporal dementia.

My interest in SUMOylation started during my postdoctoral training at Columbia University, in the lab of Nobel Laureate prof. Eric Kandel, a well-known expert in the field of memory, when I was studying the role of the prion-like protein CPEB3 in learning and memory. The observation that SUMO2 conjugation is an inhibitor constraint of the aggregation of the prion-like protein CPEB3, inspired my recent experiments on regulation of tau aggregation and toxicity in frontotemporal dementia and Alzheimer disease, which are the foundation for the present proposal.

B. Positions and Honors**Employment**

04/2002-12/2005	PhD Student, Open University, Prion Unit (directed by Dr. Roberto Chiesa), Dulbecco Telethon Institute and Mario Negri Institute for Pharmacological Research, Milan, Italy
10/2005-05/2007	Postdoctoral Research Scientist, Department of Neuroscience, Columbia University
06/2007-10/2010	Postdoctoral fellow, Howard Hughes Medical Institute
10/2010-05/2016	Associate Research Scientist, Department of Neuroscience, Columbia University
07/2016-12/2017	Consultant, Rottapharm Biotech, Monza, Italy
01/2017-Pres.	Head of Laboratory, Department of Neuroscience, Mario Negri Institute, Milan, Italy
01/2019-12/2021	Consultant, Plico Biotech, New York
04/2019-Pres.	Adjunct Associate Research Scientist, Department of Neuroscience, Columbia University
01/2021-Pres.	Associate Member, Neurobiology Lab at UC Santa Barbara (directed by Kenneth Kosik)

Honors

2001-2002	Fellowship, Mario Negri Institute for Pharmacological Research
-----------	--

2002	Scholarship grant to present at the World Alzheimer Congress, Stockholm
2002-2004	Fellowship, the University of Milan to attend post-graduate School in Biotechnology Applications
2004-2005	Fellowship, "Fondazione Monzino" for PhD students
2006-2007	Fellowship, Italian Academy for Advanced Studies at Columbia University
2009	Mentorship Award, Columbia Mentoring Initiative
2015	Telethon Career Award

Professional Societies

Society for Neuroscience (USA)
New York Academy of Science
Molecular and Cellular Cognition Society

Reviewer

Journal of Alzheimer Disease, Brain Sciences, Frontiers, Acta Neuropathologica, EMBO Journal, JBC
Alzheimer's Association

Editorial Boards

Neural Plasticity
Journal of Neuroscience Research

Advisory Boards

Yeshiva University Biotech Program
Metropolitan Montessori School Health Advisory

C. Contributions to Science

The long-term **goal of my research is to develop therapeutic anti-aggregation tools**, which eventually can be used in *in vivo* situations. The overall objective I have is to study the cellular and molecular biology and pathology of prion-like infections and to use gained understanding for **delineating novel targets** for such intervention, in particular in the context of Alzheimer's Disease and Huntington's Disease. Specifically, my most significant contributions to science are:

1. Abnormal forms of the prion protein in the pathogenesis of prion diseases: the extracellular pathway.

In my doctoral thesis, I investigated two alternative pathways by which PrP might exert a toxic effect towards neuronal cells: 1) the **extracellular pathway**, consisting of neurodegeneration induced by PrP aggregates deposition in the brain, simulated by the employment of synthetic PrP-derived peptides on primary neuronal cultures. My work demonstrated that the effect of the peptides was not mediated by abnormal PrP species and suggested that PrP peptides could interfere with a physiological function of PrP (Fioriti et al., 2007) (Fioriti et al., 2005a), (Biasini et al., 2006), (Dossena et al., 2008).

- Fioriti L**, Quaglio E, Massignan T, Stewart RS, Salmona M, Harris DA, Forloni G, Chiesa R. The neurotoxicity of prion protein (PrP) peptide 106-126 is independent of the expression level of PrP and is not mediated by abnormal PrP species. *Mol Cell Neurosci*. 2005; 28(1):165-76. PMID: 15607951
- Fioriti L**, Angeretti N, Colombo L, DeLuigi A, Tagliavini F, Salmona M, Chiesa R, Forloni G. Neurotoxic and gliotrophic activity of a synthetic peptide homologous to Gerstmann-Straussler-Scheinker disease amyloid protein. *J Neurosci*. 2007; 27(7):1576-83. PMCID: PMC6673725
- Biasini E, Massignan T, **Fioriti L**, Rossi V, Dossena S, Salmona M, Forloni G, Bonetto V, Chiesa R. Proteomic analysis of a transgenic mouse model of inherited prion disease reveals preclinical alteration of calcineurin activity. *Proteomics*. 2006; 6(9):2823-34. PMID: 16572473
- Dossena S, Imeri L, Mangieri M, Garofoli A, Ferrari L, Senatore A, Restelli E, Balducci C, Fiordaliso F, Salio M, Bianchi S, **Fioriti L**, Morbin M, Pincherle A, Marcon G, Villani F, Carli M, Tagliavini F, Forloni G, Chiesa R. Mutant prion protein expression causes motor and memory deficits and abnormal sleep patterns in a transgenic mouse model. *Neuron*. 2008; 60(4):598-609. PMID: 19038218

2. Abnormal forms of the prion protein in the pathogenesis of prion diseases: the intracellular pathway. The second line of research related to the toxicity of the prion protein is about the **intracellular pathway**, consisting of the production of aberrant and mislocalised PrP species, like transmembrane or

cytosolic PrP, induced *in vitro* by the administration of drugs interfering with maturation and PrP metabolism. As for the intracellular pathway, no evidence of cytosolic PrP was found in neurons expressing wild-type or mutant PrPs, thus arguing against the hypothesis that perturbation of PrP metabolism through the proteasomal pathway plays a pathogenic role in prion diseases. Instead my data suggested that PrP may exert a protective, anti-apoptotic function (Fioriti et al., 2005b, Orsi et al., 2006; Drisaldi et al., 2003; Restelli et al., 2010).

- a. Restelli E, **Fioriti L**, Mantovani S, Airaghi S, Forloni G, Chiesa R. Cell Type-specific Neuroprotective Activity of Untranslocated Prion Protein. *PLoS One*. 2010; 5(10):e13725. PMID: PMC2965675
- b. Orsi A, **Fioriti L**, Chiesa R, Siti R. Conditions of ER stress favour the accumulation of cytosolic PrP. *J Biol Chem*. 2006; 281(41):30431-8. PMID: 16908519
- c. **Fioriti L**, Dossena S, Stewart LR, Harris DA, Forloni G, Chiesa R. Cytosolic prion protein (PrP) is not toxic in N2a cells and primary neurons expressing pathogenic PrP mutations. *J Biol Chem*. 2005; 280(12):11320-8. PMID: 15632159
- d. Drisaldi B, Stewart RS, Adles C, Stewart LR, Quaglio E, Biasini E, **Fioriti L**, Chiesa R, Harris DA. Mutant PrP is delayed in its exit from the endoplasmic reticulum, but neither wild-type nor mutant PrP undergoes retrotranslocation prior to proteasomal degradation. *J Biol Chem*. 2003; 278 (24):21732-43. PMID:12663673

3. Structural analysis of CPEB and other polyQ and Q/N rich prions. After my PhD, I became intrigued by the possibility that a prion-like molecular switch might operate under more physiological conditions and mediate the normal function (and not just dis-function) of a protein. While Prions were initially identified as protein infectious agents causing neurological diseases by misfolding from their native conformation to a self-propagating, aggregation-prone β sheet-rich secondary structure, subsequent studies delineated a group of prions in yeast, with glutamine/asparagine-rich (Q/N-rich) domains, which are not pathogenic. During my postdoc in the Kandel lab, I found a Q/N-rich prion protein that serves a physiological function in the nervous system of mammals (Fioriti et al., 2015). The structural transitions of functional prions are not easily conceivable as an uncontrolled β sheet misfolding process. Indeed, through bioinformatics and mutagenesis studies, I found the presence of Coiled Coil (CC) domains in CPEB, Ure2 and Huntingtin protein (Fiumara et al. 2010). Unlike misfolding β -sheets, CCs can change their oligomeric state in response to physiological signals. Using structure-guided mutagenesis, we found that CC domains regulate the aggregation, insolubility, and activity of CPEB, Ure2 and huntingtin proteins. Based on these findings, we propose a model in which CCs have a critical role in the structural dynamics of functional prions and polyQ and Q/N rich proteins. These studies have continued with deletion analysis of the CPEB3 prion domain that uncovered a tripartite organization: two aggregation-promoting domains surround a regulatory module that affects interaction with the actin cytoskeleton, thus providing direct evidence that CPEB3 is a functional prion in the mammalian brain involved in synaptic plasticity and long-term memory (Stephan et al. 2015).

- a. Fiumara F, **Fioriti L**, Kandel ER, Hendrickson WA. Essential Role of Coiled Coils for Aggregation and Activity of Q/N-Rich Prions and PolyQ Proteins. *Cell*. 2010; 143:1121-35. PMID: PMC3472970
- b. Stephan J, **Fioriti L**, Lamba N, Colnaghi L, Karl K, Derkach I, Kandel ER. CPEB3 is a functional prion that interacts with the actin cytoskeleton. *Cell Rep*. 2015; 11(11):1772-85. PMID: 26074072
- c. Ford L, Ling E, Kandel ER, **Fioriti L**. CPEB3 inhibits translation of mRNA targets by localizing them to P bodies. *Proc Natl Acad Sci U S A*. 2019 Sep 3;116(36):18078-18087.
- d. Ford LK, **Fioriti L**. Coiled-Coil Motifs of RNA-Binding Proteins: Dynamicity in RNA Regulation. *Front Cell Dev Biol*. 2020; 8:607947. PMID: PMC7710910

4. CPEB3 aggregation, regulation, and activity. During my post-doc, I tested the idea that local protein synthesis, essential for the hippocampal-based perpetuation of explicit memory storage, might be regulated by a CPEB functional prion. Bacterially expressed CPEB3 forms large amyloid fibers, while CPEB3 lacking the Q/N-rich domain does not. When expressed in yeast and cell lines we found that CPEB3 has all the hallmarks of a classical prion: it forms heritable aggregates migrating as a large molecular weight, SDS-resistant smear in agarose gels, and this aggregation is dependent on the N-terminal, Q/N rich domain (Stephan et al., 2015). Moreover, the conditional knockout of CPEB3s that I generated has deficits in L-LTP (late phase of long term potentiation) and in the maintenance of long-term memory (Fioriti et al., 2015). My findings provide the first evidence for a **functional prion in the mammalian brain** and illustrate that the CPEB-mediated protein synthesis is important in the maintenance of explicit memory. Since

functional prions are potentially explosive, we were interested in knowing how they are regulated. I found that CPEB3 is dormant in the basal state and represses translation (Fioriti et al., 2015), while it is activated by synaptic activity via ubiquitination mediated by the E3 ubiquitin ligase Neuralized1 (Pavlopoulos et al., 2011). On the contrary, SUMOylation appears to mediate the translational repression operated by CPEB3, by promoting its solubilization and competing with ubiquitination (Drisaldi et al., 2015).

- a. **Fioriti L**, Myers C, Huang YY, Li X, Stephan J, Colnaghi L, Trifileff P, Drisaldi B, Pavlopoulos E, Kandel E. The Persistence of Hippocampal-based Memory Requires CPEB3-mediated Protein Synthesis. *Neuron*. 2015; 86(6):1433-48. PMID: 26074003
- b. Drisaldi B, Colnaghi L, Levine A, Huang Y, Snyder AM, Metzger DJ, Theis M, Kandel DB, Kandel ER, **Fioriti L**. Cytoplasmic Polyadenylation Element Binding Proteins CPEB1 and CPEB3 Regulate the Translation of FosB and Are Required for Maintaining Addiction-Like Behaviors Induced by Cocaine. *Front Cell Neurosci*. 2020;14:207. PMCID: PMC7365288
- c. Drisaldi B, Colnaghi L, **Fioriti L***, Meyers C, Tarasoff J, Fraser PE, Kandel ER. SUMOylation is an inhibitory constraint that regulates the prion-like aggregation and activity of CPEB3. *Cell Rep*. 2015; 11(11):1694-702. PMCID: PMC5477225 *Co-first author.
- d. Pavlopoulos E, Trifileff P, Chevaleyre V, **Fioriti L**, Zairis S, Pagano A, Malleret G, Kandel ER. Neuralized1 activates CPEB3: a function for nonproteolytic ubiquitin in synaptic plasticity and memory storage. *Cell*. 2011; 147(6):1369-83. PMCID: PMC3442370

5. Development of novel therapeutic approaches for AD and other neurodegenerative diseases.

The ultimate goal of my research is to design novel therapeutic approaches that might be effective in preventing or delaying disease onset. To this end, I more recently started to investigate the role of post-translational modifications in the modulation of aggregation, toxicity, and release of aggregation-prone proteins, such as Htt, Tau, and α -synuclein.

- a. Natale C, Barzago MM, Colnaghi L, De Luigi A, Orsini F, **Fioriti L***, Diomedea L. A Combined Cell-Worm Approach to Search for Compounds Counteracting the Toxicity of Tau Oligomers In Vivo. *Int J Mol Sci*. 2022 Sep 24;23(19):11277. doi: 10.3390/ijms231911277. PMID: 36232578; *Co-corresponding author.
- b. Orsini, E, Argyrosi, E, Restelli, L, Ford, H, Takamura, S, Matsuzaki, L, Zentilin, R, Pascente, N, M Kanaan, R, Soni, T, Katayama, R, Chiesa, G, Forloni, K, S. Kosik, ER. Kandel, PE. Fraser, O, Arancio, **Fioriti L**. SUMO2 Protects Against Tau-induced Synaptic and Cognitive Dysfunction. *bioRxiv* 2022.11.11.516192; doi: <https://doi.org/10.1101/2022.11.11.516192>
- c. Ford L, **Fioriti L**, Kandel ER. Ubiquitination and SUMOylation of Amyloid and Amyloid-like Proteins in Health and Disease. *Curr Issues Mol Biol*. 2019 Aug 18;35:195-230.
- d. Hayashi Y, Ford LK, **Fioriti L**, McGurk L, Zhang M. Liquid-liquid phase separation in physiology and pathophysiology of the nervous system *J Neurosci*, 41 (2021), pp. 834-844

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/pubmed/?term=Fioriti+Luana>