

BIOGRAPHICAL SKETCH

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NAME: Michael Assfalg

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POSITION TITLE: Full Professor

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 (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Florence, Italy	MSc	12/1997	Chemistry
University of Florence, Italy	PhD	12/2001	Biomolecular NMR sp.
University of Florence, Italy	Postdoc	10/2002	Protein science
University of Maryland, USA	Postdoc	04/2004	Structural biology
University of Frankfurt, Germany	Postdoc	01/2006	Biomolecular and medicinal chemistry

A. Personal Statement

I am a Full Professor of Organic Chemistry in the Department of Biotechnology at the University of Verona. I earned my PhD in Chemistry from the University of Florence in 2001, followed by postdoctoral training at the University of Maryland (2002–2004) under the supervision of Prof. Fushman, where I investigated the structure and dynamics of polyubiquitin chains. I subsequently held a Marie Curie postdoctoral fellowship for Transfer of Knowledge at the University of Frankfurt (2004–2005), focusing on protein–drug interactions.

After joining the University of Verona in 2006, I established my independent research group in 2012. My research is centered on the structure and chemistry of biologically relevant molecules and their roles in health and disease. In particular, my group studies molecular interactions at atomic resolution, with a strong emphasis on proteins implicated in neurodegenerative disorders, including Parkinson's and Alzheimer's disease. Current projects address how post-translational modifications regulate the aggregation and misfolding of amyloidogenic proteins such as α -synuclein and tau. In parallel, we develop innovative chemical and biophysical strategies, including nanoparticle-based approaches, to modulate or prevent pathological protein aggregation.

I have served as Principal Investigator on research projects funded through local, national, and international programs and have established collaborations with research groups in the United States, Greece, France, Finland, and Italy. I have authored over 90 peer-reviewed publications in international journals and books and have delivered invited and contributed oral presentations at national and international scientific meetings.

My work integrates chemical biology, structural biophysics, and molecular neuroscience to elucidate the fundamental mechanisms underlying neurodegenerative diseases. I bring extensive experience in leading interdisciplinary research teams, mentoring graduate students and postdoctoral fellows, and translating methodological advances into biological insights. Through these efforts, I aim to make rigorous and collaborative contributions to research programs focused on protein post-translational modifications, biomolecular interactions, protein aggregation, and neurodegeneration.

B. Positions, Scientific Appointments, and Honors

Positions:

10/2023-present Full Professor of Organic Chemistry, Univ. of Verona, Dept. of Biotechnology

11/2014-9/2023 Associate Professor of Organic Chemistry, Univ. of Verona, Dept. of Biotechnology

01/2006-10/2014 Assistant Professor of Organic Chemistry, Univ. of Verona, Dept. of Biotechnology

09/2004-12/2005 Postdoc, University of Frankfurt, Germany

05/2004-08/2004 Senior Scientist, ProtEra s.r.l., spin-off company

11/2002-04/2004 Postdoc, University of Maryland, Dept. of Chemistry and Biochemistry, USA

02/2002-10/2002 Postdoc, University of Florence, Center of Magnetic Resonance

01/1999-12/2001 PhD student, University of Florence, Center of Magnetic Resonance

Appointments:

2021 - 2023 Elected President of the Division of Chemistry of Biological Systems of the Italian Chemical Society

2012 - 2020 Elected Member of the Board of Directors of the Division of Chemistry of Biological Systems of the Italian Chemical Society

C. Contributions to Science

1) During my postdoc at the University of Maryland, I worked on understanding the conformational properties of distinct polyubiquitin chains and their interactions with characteristic domains of their cognate proteins. In fact, at the time, these systems were still poorly characterized at the molecular level, and the conclusions of various studies appeared rather controversial. Fortunately, in collaboration with Cecile Pickart, we were able to produce ubiquitin polymers with the desired linkages (Lys48- or Lys63-) and to develop various techniques based on NMR spectroscopy that allowed us to determine their structure in solution. We indeed provided the first experimental evidence that alternatively linked polyubiquitin chains adopt distinct conformations in solution. These results provided the basis for understanding the determinants of the different substrate recognition specificities by distinct polyubiquitin types.

- a. Varadan, R.; **Assfalg, M.**; Haririnia, A.; Raasi, S.; Pickart, C.; Fushman, D. Solution Conformation of Lys63-Linked Di-Ubiquitin Chain Provides Clues to Functional Diversity of Polyubiquitin Signaling. *J. Biol. Chem.* 2004, 279, 7055–7063, doi:10.1074/jbc.M309184200.
- b. Varadan, R.; **Assfalg, M.**; Raasi, S.; Pickart, C.; Fushman, D. Structural Determinants for Selective Recognition of a Lys48-Linked Polyubiquitin Chain by a UBA Domain. *Mol. Cell* 2005, 18, 687–698, doi:10.1016/j.molcel.2005.05.013.
- c. Fushman, D.; Varadan, R.; **Assfalg, M.**; Walker, O. Determining Domain Orientation in Macromolecules by Using Spin-Relaxation and Residual Dipolar Coupling Measurements. *Prog. Nucl. Magn. Reson. Spectrosc.* 2004, 44, 189–214, doi:10.1016/j.pnmrs.2004.02.001.
- d. Varadan, R.; **Assfalg, M.**; Fushman, D. Using NMR Spectroscopy to Monitor Ubiquitin Chain Conformation and Interactions with Ubiquitin-Binding Domains. *Methods Enzymol.* 2005, 399, 177–192, doi:10.1016/S0076-6879(05)99012-5.

2) The activity of many proteins is intimately linked to interactions with lipids as isolated molecules or in the form of biological membranes. Understanding how these macromolecules interface with lipid surfaces has been a constant point of attention for me. My laboratory was able to describe at the sub-molecular level how soluble and cytosolic proteins interact transiently with membranes. Even more significant is the impact that lipids have on amyloid protein aggregation, a topic of great interest nowadays. In this respect, we have provided novel insights into the mechanism of lipid-mediated tau aggregation.

- a. Ceccon, A.; D'Onofrio, M.; Zanzoni, S.; Longo, D.L.; Aime, S.; Molinari, H.; **Assfalg, M.** NMR Investigation of the Equilibrium Partitioning of a Water-Soluble Bile Salt Protein Carrier to Phospholipid Vesicles. *Proteins* 2013, 81, 1776–1791, doi:10.1002/prot.24329.
- b. Ceccon, A.; Lelli, M.; D'Onofrio, M.; Molinari, H.; **Assfalg, M.** Dynamics of a Globular Protein Adsorbed to Liposomal Nanoparticles. *J. Am. Chem. Soc.* 2014, 136, 13158–13161, doi:10.1021/ja507310m.
- c. Barracchia, C.G.; Tira, R.; Parolini, F.; Munari, F.; Bubacco, L.; Spyroulias, G.A.; D'Onofrio, M.; **Assfalg, M.** Unsaturated Fatty Acid-Induced Conformational Transitions and Aggregation of the Repeat Domain of Tau. *Molecules* 2020, 25, 2716, doi:10.3390/molecules25112716.

3) Both experiments and theory support the view that macromolecular crowding and confinement exert profound quantitative effects on macromolecular interactions in living systems. Macromolecular crowding and confinement together cause restricted translational diffusion inside cells; they influence the energetics of associations and may stimulate protein aggregation. In my work, I have studied molecular phenomena in a context that mimics the cellular environment, introducing macromolecular cosolutes, lipid membrane models, or directly observing molecular properties in cell extracts. This approach was used in particular to explain the association of ubiquitin with cognate binding domains. These concepts enabled me to discover behaviors that were unobservable in dilute solutions, also allowing me to contribute to the development of artificial cells. The acquired knowledge is particularly useful in view of my current studies on biomolecular condensates, where crowding is a distinctive factor.

- a. Ceccon, A.; Busato, M.; Pérez Santero, S.; D'Onofrio, M.; Musiani, F.; Giorgetti, A.; **Assfalg, M.** Transient Interactions of a Cytosolic Protein with Macromolecular and Vesicular Cosolutes: Unspecific and Specific Effects. *ChemBioChem* 2015, 16, 2633–2645, doi:10.1002/cbic.201500451.
- b. Pérez Santero, S.; Favretto, F.; Zanzoni, S.; Chignola, R.; **Assfalg, M.**; D'Onofrio, M. Effects of Macromolecular Crowding on a Small Lipid Binding Protein Probed at the Single-Amino Acid Level. *Arch. Biochem. Biophys.* 2016, 606, 99–110, doi:10.1016/j.abb.2016.07.017.
- c. Munari, F.; Bortot, A.; Zanzoni, S.; D'Onofrio, M.; Fushman, D.; **Assfalg, M.** Identification of Primary and Secondary UBA Footprints on the Surface of Ubiquitin in Cell-Mimicking Crowded Solution. *FEBS Lett.* 2017, 591, 979–990, doi:10.1002/1873-3468.12615.
- d. Lentini, R.; Santero, S.P.; Chizzolini, F.; Cecchi, D.; Fontana, J.; Marchioretto, M.; Del Bianco, C.; Terrell, J.L.; Spencer, A.C.; Martini, L.; Forlin, M.; **Assfalg, M.**; et al. Integrating Artificial with Natural Cells to Translate Chemical Messages That Direct E. Coli Behaviour. *Nat. Commun.* 2014, 5, 4012, doi:10.1038/ncomms5012.

4) Recent years have witnessed enormous efforts to investigate interactions between biomolecules and engineered nanomaterials, driven by the realization that these devices offer unprecedented capabilities as tools in biomedical research, diagnostics, and therapy. Two possible applications immediately appeared very attractive to me, namely the development of nanoparticles as artificial receptors for the specific recognition of target proteins and nanoparticles acting as nanochaperones to mitigate the formation of toxic aggregates associated with proteinopathies.

We have been able to describe the interaction modes of some proteins with simple nanoparticles, demonstrating that these nanosystems can interact specifically with biomolecular recognition sites of

(poly)ubiquitin or modulate the conformational space of disordered amyloid proteins, opening new opportunities for the development of diagnostic and therapeutic tools.

- a. Zanzoni, S.; Ceccon, A.; **Assfalg, M.**; Singh, R.K.; Fushman, D.; D'Onofrio, M. Polyhydroxylated [60]Fullerene Binds Specifically to Functional Recognition Sites on a Monomeric and a Dimeric Ubiquitin. *Nanoscale* 2015, 7, 7197–7205, doi:10.1039/C5NR00539F.
- b. Zanzoni, S.; Pedroni, M.; D'Onofrio, M.; Speghini, A.; **Assfalg, M.** Paramagnetic Nanoparticles Leave Their Mark on Nuclear Spins of Transiently Adsorbed Proteins. *J. Am. Chem. Soc.* 2016, 138, 72–75, doi:10.1021/jacs.5b11582.
- c. Bortot, A.; Zanzoni, S.; D'Onofrio, M.; **Assfalg, M.** Specific Interaction Sites Determine Differential Adsorption of Protein Structural Isomers on Nanoparticle Surfaces. *Chem. - Eur. J.* 2018, 24, 5911–5919, doi:10.1002/chem.201705994.
- d. Tira, R.; De Cecco, E.; Rigamonti, V.; Santambrogio, C.; Barracchia, C.G.; Munari, F.; Romeo, A.; Legname, G.; Prosperi, D.; Grandori, R.; **Assfalg, M.** Dynamic Molecular Exchange and Conformational Transitions of Alpha-Synuclein at the Nano-Bio Interface. *Int. J. Biol. Macromol.* 2020, 154, 206–216, doi:10.1016/j.ijbiomac.2020.03.118.

5) Recently, we have turned our efforts to decipher the role of ubiquitin and ubiquitination in the phenomenon of protein aggregation associated with neurodegenerative diseases. Specifically, an increasing number of studies have led us to consider the possible effects of ubiquitination on the pathological conformational transitions of tau. Indeed, it has been proposed that ubiquitination, together with other PTMs, can play a decisive role in directing the formation of specific forms of aggregates and insoluble filaments. Using a combination of semisynthetic protein chemistry, enzymatic ubiquitination, and various biophysical approaches, we have shown that ubiquitination directly modulates tau aggregation and liquid–liquid phase separation in a site- and cofactor-dependent manner. I contributed to elucidating how specific ubiquitin conjugation patterns alter tau conformational ensembles and aggregation pathways, linking post-translational modification to neurodegenerative processes. In parallel, my work provided structural insight into the chaperone-independent ubiquitination of tau by the E3 ligase CHIP, revealing the molecular basis of tau recognition and modification. More recently, I established a multidisciplinary structural biology framework integrating native ion mobility mass spectrometry, SAXS, scaled molecular dynamics simulations, and NMR spectroscopy to resolve the highly dynamic conformational ensemble of the amyloid-forming tau and to quantify its remodeling induced by ubiquitin modification, highlighting the mechanistic role of ubiquitination in tau conformational transitions.

- a. Munari, F.; Barracchia, C.G.; Franchin, C.; Parolini, F.; Capaldi, S.; Romeo, A.; Bubacco, L.; **Assfalg, M.**; Arrigoni, G.; D'Onofrio, M. Semisynthetic and Enzyme-Mediated Conjugate Preparations Illuminate the Ubiquitination-Dependent Aggregation of Tau Protein. *Angew. Chem. Int. Ed.* 2020, 59, 6607–6611, doi:10.1002/anie.201916756.
- b. Parolini F, Tira R, Barracchia CG, Munari F, Capaldi S, D'Onofrio M, **Assfalg M.** Ubiquitination of Alzheimer's-related tau protein affects liquid-liquid phase separation in a site- and cofactor-dependent manner. *Int J Biol Macromol.* 2022 Mar 15;201:173-181. doi: 10.1016/j.ijbiomac.2021.12.191.
- c. Munari F, Mollica L, Valente C, Parolini F, Kachoe EA, Arrigoni G, D'Onofrio M, Capaldi S, **Assfalg M.** Structural Basis for Chaperone-Independent Ubiquitination of Tau Protein by Its E3 Ligase CHIP. *Angew Chem Int Ed Engl.* 2022 Apr 4;61(15):e202112374. doi: 10.1002/anie.202112374.
- d. Viola G, Trivellato D, Laitaoja M, Jänis J, Felli IC, D'Onofrio M, Mollica L, Giachin G, **Assfalg M.** Conformational signatures induced by ubiquitin modification in the amyloid-forming tau repeat domain. *Proc Natl Acad Sci U S A.* 2025 Apr 15;122(15):e2425831122. doi: 10.1073/pnas.2425831122.