

BIOSKETCH

NAME: Giovanna Musco

POSITION TITLE: Group Leader, Ph.D

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Milano)	BSc-MA in Chemistry Final mark: 108/110	07/1992	Biochemistry and Structural biology
EMBL Heidelberg	-	06/1993	Biochemistry and Structural Biology,
EMBL Heidelberg	Ph.D Summa cum Laude	05/1998	Biochemistry, and structural biology

A. Personal Statement

I am a structural biologist, my basic training is in Chemistry and I have been trained in Biochemistry and Structural biology in several internationally recognized European research institutes (EMBL-Heidelberg; National Institute for Medical Research-London, Biozentrum-Basel). I use biomolecular Nuclear Magnetic Resonance (NMR) coupled to biochemical, biophysical and computational methods to study the structure/function activity of proteins (or protein domains) involved in human diseases (genetic diseases and cancer) and their interactions with other ligands (protein and/or small molecules). I also apply NMR as analytical tool in Metabolomics. Since 2001, thanks to a Telethon Career award, I work as independent PI as Head of the Biomolecular NMR Laboratory at IRCCS San Raffaele. Since 2016 I am Adjunct Professor in General Chemistry at the Vita-Salute San Raffaele University, Milano Italy.

During these years I have been PI in several grants, I successfully administered several research projects and collaborated with other researchers within and outside my research Institute, where my expertise in Structural Biology and Biochemistry was fundamental in giving insights into the molecular determinants of human disease (cancer, genetic diseases). I have also collaborated with a Biotech Company (MolMed), where my expertise in NMR in drug-discovery was highly appreciated. I am aware of the importance of building a realistic research plan (including feasibility, back-up strategies, timeline, and budget) and I ground my research on frequent communication among project members and collaborators. As a result, as independent researcher, I produced several peer-reviewed publications from each project.

B. Positions, Scientific Appointments, and Honors

Since 2013	Permanent Position as Head of Biomolecular NMR Laboratory at Ospedale, Milano-Italy
2010-2013	Tenure-Track appointment as Head of Biomolecular NMR Unit at Ospedale San Raffaele, Milano-Italy
2008-2013	Telethon Associate Scientist at IRCCS San Raffaele, Milano-Italy
2006-2013	Scientific consultant of the biotech-company Molmed SpA Milano-Italy
2001- 2007	Telethon Assistant Scientist at IRCCS San Raffaele, Milano-Italy
2001- 2007	Head of Biomolecular NMR laboratory at IRCCS San Raffaele, Milano-Italy
2000-2001	EMBO post-doctoral fellow at the Biozentrum, Basel-CH

1998-1999	Research associate in the NMR laboratory of the Division of Molecular Structure, National Institute for Medical Research, Mill Hill, London.
TEACHING EXPERIENCES	
2020-2023	Adjunct Professor of NMR in metabolomics (Master of Bioinformatics and functional genomics, Università degli Studi di Milano)
2019	Italian habilitation Full Professor in General Chemistry (CHIM03)
2019	Italian habilitation Full Professor in Biochemistry (BIO10)
Since 2016	Adjunct Professor of General Chemistry (B.Sc. Course in Biotechnology) at Università Vita-Salute San Raffaele-Milan, Italy
2014-2015	Lecturer, NMR: principles and applications to the study of protein-ligand interactions and metabolomics, B.Sc. Course in Biotechnology, Università Vita-Salute San Raffaele-Milan Italy
2015-2024	Lecturer on Biomolecular NMR at International PhD program at SEMM IEO Milan, Italy
2015	Lecturer on Biomolecular NMR at International PhD program at University of Brescia, Italy
2007	Lecturer on Biomolecular NMR at International PhD program at IFOM. Milan, Italy
SUPERVISION ACTIVITY	
I have supervised 13 Master students, 8 Ph.D students and 12 Post-Docs	
HONORS AND AWARDS	
1992-1993	Fellowship by the University of Milan for training abroad
1992-1994	Deutscher Akademischer Austausch Dienst (DAAD) fellowship
1994-1998	EU training fellowship
2000-2001	EMBO long term post-doctoral fellowship
2001-2007	Telethon Assistant Scientist Award
2007	EMBO short-term fellowship at EMBL-Heidelberg
2007-2013	Telethon Associate Scientist Award
REVIEWER FOR JOURNALS	
Cell Reports, Nucleic Acids Research, Scientific Reports, Plos Computation, TIBS, Chemistry, Journal of American Chemical Society, Journal of Biological Chemistry, Journal of Molecular Biology, BMC-Biology, Nature Communications	
REVIEWER FOR FUNDING AGENCIES AND ACADEMY	
2024	Member of the evaluation panel ICREA (Catalan Institution for Research and Advanced Studies) Call 2024 Life&Medical Sciences.
2023	External scientific reviewer for Research Foundation Flandern
2022	Member of the evaluation panel for ERC-Consolidators grant LS1
2022	External scientific reviewer for Research Foundation Flandern
2021	Examiner for Ph.D Thesis: Structural, dynamics and functional study of the phosphorylation of BAF protein: a dimer mutated in premature ageing syndromes (Candidate Sophie Marcelot, Université Paris-Saclé)
2020	Member of the evaluation panel for ERC-Consolidators grant LS1
2020	Examiner for Ph.D Thesis: Methods for the Biochemical and Structural Characterization of the NADPH Oxidase 5 (IUSS-Scuola Universitaria Superiore -Pavia)
2020	Examiner for Ph.D Thesis: Metabolomics and Bladder Cancer Risk factors and prognosis of the most common cancer of the urinary tract (Università Roma Tor Vergata)
2019	External Reviewer for ERC-Consolidators grant LS1
2018	Member of the evaluation panel for ERC-Consolidators grant LS1

2017	Member of the Interdisciplinary Panel of Experts for the International Research Agendas Program, Polish Academy of Science
2017	External Reviewer for Agence National de La Recherche (France)
2016	External Reviewer for Israel Science Foundation
2016	External Reviewer for Anvur (Italian National Agency for University Evaluation)
2016	Member of the evaluation panel for ERC-Consolidators grant LS1
2015	External scientific reviewer for Research Foundation Flandern
2014	External Reviewer for Agence National de La Recherche (France)
2014	Examiner for Ph.D Thesis: Structural Insights into Substrate Binding and Regulation of E3 Ubiquitin Ligases in the Nedd4 Family using NMR Spectroscopy
2013	External scientific reviewer for Research Foundation Flandern

C. Contributions to Science

C1. As Ph.D. student at EMBL-Heidelberg I determined the first solution structure of the KH domain, a sequence motif found in several RNA-binding proteins. This work gave the first atomistic rationale for the structural/functional effects of pathological mutations within the KH domain of FMRP, the protein involved in Fragile X mental retardation (1). I also solved the structure of frataxin and rationalized the effects of the pathological point-mutations that give rise to the genetic disease Friedreich ataxia (2) As EMBO post-doctoral fellow at the Biozentrum (Basel, CH) I gave methodological contributions to the NMR technique developing a method to measure relevant NMR structural parameters (3). The method is now routinely applied by the NMR community.

C.2 Best achievements as PI at IRCCS Ospedale San Raffaele (OSR):

In 2001 I started my independent career at OSR as Telethon Assistant Scientist (upgraded than to Telethon Associate Scientist). With this grant I paid my salary and part of my research for 13 years. Since 2013 I am Head of Biomolecular NMR Laboratory, Ospedale San Raffaele, Milano-Italy.

The Telethon career award allowed me to introduce Structural Biology, Biomolecular NMR and Computational Biology (Molecular Dynamics Simulations, Docking, Virtual screening) in the Institute. This expertise was not present at OSR. Thanks to the Telethon support I gave important contributions to the structural/functional characterization of PHD fingers as epigenetic readers in genetic diseases and thanks to other grants and to a collaboration with the biotech company Molmed SpA I contributed to the identification/structural characterization of different protein-protein, protein-drug complexes.

C.2.1. Epigenetic readers. Epigenetic regulation of gene transcription relies on structural domains able to recognize post-translational modifications (PTM) on histones. PTMs misinterpretation is often associated with disease conditions. Herein PHD fingers, small Zn²⁺ binding domains, represent a paradigmatic example, as their mutations are associated to cancer or genetic diseases. We studied the structure/function relationship of the PHD fingers of AIRE (Autoimmune Regulator), the protein responsible of the autoimmune disease APECED (Autoimmune polyendocrinopathy ectodermal dystrophy). AIRE is a transcriptional regulator expressed in thymic medullary epithelial cells where it promotes the expression of tissue-specific antigens. In collaboration with P. Peterson (Uni-Tartu), we were the first to demonstrate that AIRE binds to non-methylated histone H3 through its first PHD finger, herewith activating gene expression. We provided the first structural rationale for why APECED causing mutations affect the transcriptional activity of AIRE (4) We also contributed to the concept that PHD fingers are not only epigenetic readers, but that they can work as structural hubs for multiple interactions (5,6). We have identified by an in silico approach combined to NMR first small molecules able to weaken the interaction between two Zn-binding domain (PHD_vC5HCHNSD1 and C2HRNizp1) of two chromatin related proteins, NSD1 and Nizp1 (7).

C.2.2. Protein-Protein, Protein-drug interactions

a. *HMGB1 inhibitors*: I identified the first small molecule able to inhibit the pro-inflammatory activity of the alarmin HMGB1 (8). I consider this one of my best and most useful achievements as independent researcher (466 cit), because herewith I provided the HMGB1 community with a convenient and effective chemical probe that is routinely used in HMGB1 functional studies. We have recently identified Diflunisal as an effective inhibitor of HMGB1/CXCL12 heterocomplex that blocks immune cell recruitment (9) Testing of this molecule against inflammation related tumours in vivo are currently ongoing in our lab. We recently **disentangled the**

molecular details of the inflammation related heterocomplex HMGB1/CXCL12 using a combination of NMR and biophysical methods demonstrating that it forms a fuzzy complex (10). This work, from the methodological point of view is of interest for this project, as it demonstrates that we master the tools and competences to study dynamic interactions

b. *Integrins inhibitors* The isoDGR motif is an integrin $\alpha\beta3$ binding motif holding promise in antiangiogenic cancer therapy. We developed an NMR method and applied new computational approaches to study the conformation (11) and interaction (12) of small isoDGR cyclopeptides with integrin $\alpha\beta3$. Most importantly, we introduced the new concept that isoDGR based cyclopeptides, at variance to classical RGD based peptides, are real antagonist of $\alpha\beta3$, blocking receptor allosteric activation (13). Of interest for this project, we have also characterized the interaction of modified peptides (e.g. stapled peptides) with other integrins using a combination of NMR and computational methods (14).

c. *Prion proteins inhibitors* We have recently identified a tetracationic porphyrin that binds to two distinct prion PrPC regions eliciting a dual anti-prion effect, i.e. inhibition to scrapie conversion and lysosomal degradation. (15)

C.2.3. NMR Metabolomics: I introduced in the hosting Institute the application of NMR as analytical tool in metabolomics. NMR metabolomics was fundamental to demonstrate a defective glucose metabolism pathway in the generic disease polycystic kidney disease and to identify a new therapeutic strategy (16), and to understand basic mechanism of cilia function (17). This work has been done in collaboration with Dr. A Boletta (IRCCS Ospedale San Raffaele)

Overall these scientific achievements testify my experience in structural biology and in the application and development of biophysical and computational methods to identify and characterize protein-ligand and protein-drug interactions to investigate the molecular mechanisms of human diseases and of their cure, including genetic ones.

D. Relevant Publications

Complete list of Published Work (97 publications, H=36) (scopus)

<https://pubmed.ncbi.nlm.nih.gov/?term=musco+g>

- (1) Three-dimensional structure and stability of the KH domain: Molecular insights into the fragile X syndrome, Musco, G et al, Cell, 1996 85 (2), pp. 237-245.
- (2) Towards a structural understanding of Friedreich's ataxia: Solution structure of frataxin Musco, G. et al Structure, 2000, 8(7), pp. 695–707
- (3) Solution NMR of proteins within polyacrylamide gels. Sass, H.-J., Musco, G et al, J. of Biom NMR, 2000
- (4) The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression Org, T., et al., Musco*, G., Peterson, P. EMBO Rep, 2008 9 (4), pp. 370-376 *corresponding author
- (5) AIRE-PHD fingers are structural hubs to maintain the integrity of chromatin-associated interactome. Gaetani, M. et al and Musco, G Nucleic Acids Research 2012, 40 (22), pp. 11756-11768
- (6) Structural basis for PHD_VC5HCH_{NSD1}-C2HRNizp1 interaction: Implications for Sotos syndrome Berardi et al. and Musco G. Nucleic Acids Research, 2016, 44 (7), pp. 3448-3463.
- (7) In silico derived small molecules targeting the finger-finger interaction between the histone lysine methyltransferase NSD1 and Nizp1 repressor Berardi et al, and Musco G. Computational and Structural Biotechnology Journal 2020 18, pp. 4082-4092
- (8) Glycyrrhizin Binds to High-Mobility Group Box 1 Protein and Inhibits Its Cytokine Activities, Mollica, et al, Musco, G*, Bianchi, M.E., Chemistry and Biology 2007 14 (4), pp. 431-441 (co-last author)
- (9) De Leo, F et al, and Musco, G. Diflunisal targets the HMGB1/CXCL12 heterocomplex and blocks immune cell recruitment (2019) EMBO Reports, 20 (10), art. no. e47788
- (10) The acidic intrinsically disordered region of the inflammatory mediator HMGB1 mediates fuzzy interactions with CXCL12 Mantonicio, MV et al, and Musco, G (2024) Nature Communications 15, Article number: 1201.
- (11) Use of metadynamics in the design of isoDGR-based $\alpha\beta3$ antagonists to fine-tune the conformational ensemble Spitaleri, A. et al. Angewandte Chemie 2011, 123 (8):1832-6

- (12) 2D TR-NOESY experiments interrogate and rank ligand-receptor interactions in living human cancer cells Mari et al, and Musco, G. Angewandte Chemie 2010 49(6):1071-4.
- (13) Molecular dynamics reveal that isoDGR-containing cyclopeptides are true $\alpha\beta 3$ antagonists unable to promote integrin allostery and activation. Ghitti, M et al., and Musco, G. Angewandte Chemie 2012
- (14) A stapled chromogranin A-derived peptide is a potent dual ligand for integrins $\alpha\beta 6$ and $\alpha\beta 8$. Nardelli F, et al, Musco G. 2019 55(98):14777-14780 and patent: WO EP US AU CA CA3158422A1
- (15) A tetracationic porphyrin with dual anti-prion activity Masone et al, and Musco G, and Chiesa R. iScience 2023 26(9),107480
- (16) Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy, Row et al, Musco G*, Boletta A. Nature Medicine, 2013. Cit. 365).
- (17) Primary cilia sense glutamine availability and respond via asparagine synthetase Steidel et al. Nat Metab. 2023, 5(3):385-397).

Giovanna Musco

Autorizzo il trattamento dei miei dati personali ai sensi della legge 675/96 per le finalità di ricerca e selezione del personale