

BIOGRAPHICAL SKETCH

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NAME: Stefano Ricagno

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

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)	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Pavia (Pavia, Italy)	Master	10/1994	07/1999	Biological Sciences
Karolinska Institute (Stockholm Sweden)	PhD	04/2000	09/2004	Medical Biochemistry and Biophysics
Architecture et Fonction des Macromolécules Biologiques, CNRS (Marseille, France)	Postdoc	11/2004	04/2006	Structural biology
Dept. of Biomolecular Sciences and Biotechnology, 10/2010 University of Milan	Postdoc	05/2006	10/2010	Structural biology and Biophysics

A. Personal Statement

Prof. S. Ricagno (ORCID number 0000-0001-6678-5873) is a structural biologist and a biophysicist with a particular interest in the structural aspects of protein misfolding and aggregation. In the last 18 years he has been actively working on many models of protein aggregation: on disease relevant amyloidogenic proteins such as Beta-2 microglobulin, Light chains, Transthyretin and on other polymerogenic proteins such as serpins (Neuroserpin, Alpha-1 Antithrombin), publishing milestone papers on these topics. The molecular determinants of protein aggregation, the structures of native and aggregated proteins, the protein aggregation and its toxicity in disease are his main subjects of study.

Lately his research is trying to close the gap between the biochemistry and structural biology *in vitro* and clinical biochemistry: *ex vivo* folded and misfolded proteins and aggregates have been successfully characterized and their molecular and structural properties unveiled¹⁻⁵.

References:

- 1 Nevone, A. *et al.* An N-glycosylation hotspot in immunoglobulin kappa light chains is associated with AL amyloidosis. *Leukemia* **36**, 2076-2085, doi:10.1038/s41375-022-01599-w (2022).
- 2 Oberti, L. *et al.* Concurrent structural and biophysical traits link with immunoglobulin light chains amyloid propensity. *Sci Rep* **7**, 16809, doi:10.1038/s41598-017-16953-7 (2017).
- 3 Schulte, T. *et al.* Cryo-EM structure of *ex vivo* fibrils associated with extreme AA amyloidosis prevalence in a cat shelter. *Nature communications* **13**, 7041, doi:10.1038/s41467-022-34743-2 (2022).
- 4 Swuec, P. *et al.* Cryo-EM structure of cardiac amyloid fibrils from an immunoglobulin light chain AL amyloidosis patient. *Nature communications* **10**, 1269, doi:10.1038/s41467-019-09133-w (2019).

5 Hofbauer, D. *et al.* beta(2)-microglobulin triggers NLRP3 inflammasome activation in tumor-associated macrophages to promote multiple myeloma progression. *Immunity* **54**, 1772-1787 e1779, doi:10.1016/j.immuni.2021.07.002 (2021).

Grants:

Over the years Ricagno's research has been funded by the Italian Ministry of research (FIRB in 2010; PRIN 2020), by the University of Milan (Linea 2: 2014, 2015, 2020; Seed4Innovation 2025) and by private Italian charities: Telethon Foundation (Research Grant, 2014 and 2017, 2024), by CARIPLO Foundation (CARIPLO Giovani 2016, 2024) and ARISLA Foundation (Research Grant, 2018), Anicura research Grant (2021), Baggi-Sisini Foundation (2021), Fondazione Veronesi (2022 and 2023 to a member of the lab, Cristina Visentin), AIRC (Investigator grant 2024).

Statistics:

Total number of publications 90 (Scopus).

H-index 28 (Scopus).

Total number of citations: 2600 (Scopus).

Structures deposited in the Protein Database: 93 (Protein Databank).

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

Academic

- Since 10/2024: Director of the PhD School in Molecular Biology of the Cell, University of Milan (Italy).
- Since 04/2022: Full Professor of Biochemistry at Dept. of Biosciences, University of Milan (Italy).
- 03/2015 - 03/2022: Associate Professor of Biochemistry at Dept. of Biosciences, University of Milan (Italy).
- Since 2013: Member of the PhD School in Molecular and Cell Biology, University of Milan (Italy).
- 11/2010 - 02/2015: Assistant professor at Dept. of Bioscience, University of Milan (Italy).

Editorial

- Since 02/2017: Member of the Editorial Board of Scientific Reports. <https://www.nature.com/srep/>

Scientific memberships

- Member of the International Myeloma Society (IMS)
- Member of the International Society of Amyloidosis (ISA)
- Member of the Italian Society of Biochemistry (SIB)
- Member of the Italian Association of Crystallography (AIC)

Other

- 02/2020 – 02/2023: Member of the Scientific Advisory Board of DrenBio (San Francisco USA). <https://www.drenbio.com>
- 10/2021 – 06/2024: Group Leader of the Structural Biology Lab at the Institute for Molecular and Translational Cardiology (San Donato Hospital, Milan, Italy). <https://www.grupposandonato.it/strutture/policlinico-san-donato>

Reviewer for the following scientific journals (last five years):

ACS Chemical Biology, Scientific Reports, Oncotarget, Amyloid, J Am. Chem Soc., Frontiers NeuroScience, IUBMB Life, J Phys Chem, Cell Mol. Biol., PlosOne, AJRCMB, Chemical Biology and Drug Design, J. of Molecular Biology, Biochemistry, Nature Communications, Science Advances.

Referee for the following grant institutions:

ERC (EU), Deutsche Forschungsgemeinschaft (Germany), Alzheimer Forschung (Germany), Italian Ministry of Research (Italy).

Member of the Independent Review Board for INSTRUCT-ERIC and CanSERV (EU).

C. Contributions to Science

1. Early scientific work (2002 - 2007)

My scientific focus during my PhD and my first postdoc was to unravel the relationship between protein structure and enzymatic mechanism in several bacterial and viral enzymes. The main technique used was X-ray crystallography.

Main publications:

- 1 Jonsson, S., Ricagno, S., Lindqvist, Y. & Richards, N. G. Kinetic and mechanistic characterization of the formyl-CoA transferase from *Oxalobacter formigenes*. *J Biol Chem* 279, 36003-36012, doi:10.1074/jbc. (2004).
- 2 Ricagno, S. et al. Crystal structure of the receptor-binding protein head domain from *Lactococcus lactis* phage bIL170. *J Virol* 80, 9331-9335, doi:10.1128/JVI.01160-06 (2006).
- 3 Ricagno, S. et al. Crystal structure and mechanistic determinants of SARS coronavirus nonstructural protein 15 define an endoribonuclease family. *Proc Natl Acad Sci U S A* 103, 11892-11897, doi:10.1073/pnas.0601708103 (2006).
- 4 Ricagno, S. et al. Crystal structure of 1-deoxy-d-xylulose-5-phosphate reductoisomerase from *Zymomonas mobilis* at 1.9-Å resolution. *Biochim Biophys Acta* 1698, 37-44, doi:10.1016/j.bbapap.2003.10.006 (2004).
- 5 Ricagno, S., Jonsson, S., Richards, N. & Lindqvist, Y. Formyl-CoA transferase encloses the CoA binding site at the interface of an interlocked dimer. *EMBO J* 22, 3210-3219, doi:10.1093/emboj/cdg333 (2003).
- 6 Schutz, A. et al. Crystal structure of thiamindiphosphate-dependent indolepyruvate decarboxylase from *Enterobacter cloacae*, an enzyme involved in the biosynthesis of the plant hormone indole-3-acetic acid. *Eur J Biochem* 270, 2312-2321, (2003).

2. Neuroserpin and FENIB (2009 – 2021)

Neuroserpin (NS) is a member of the serine protease inhibitors superfamily. Specific point mutations are responsible for its polymerization and accumulation in the endoplasmic reticulum of neurons that leads to an incurable progressive dementia named familial encephalopathy with neuroserpin inclusion bodies (FENIB). Over the years, I have been working on characterizing the structure of native and of aggregated NS. Detailed molecular studies unveiled the biophysical properties of pathologic NS polymerisation. Moreover, we identified a small molecule which prevents NS polymerization in vitro.

Main publications:

- 1 Noto, R. et al. The tempered polymerization of human neuroserpin. *PLoS One* 7, e32444, doi:10.1371/journal.pone.0032444 (2012).
- 2 Ricagno, S., Caccia, S., Sorrentino, G., Antonini, G. & Bolognesi, M. Human neuroserpin: structure and time-dependent inhibition. *J Mol Biol* 388, 109-121, doi: 10.1016/j.jmb.2009.02.056 (2009).
- 3 Ricagno, S. et al. Two latent and two hyperstable polymeric forms of human neuroserpin. *Biophys J* 99, 3402-3411, doi:10.1016/j.bpj.2010.09.021 (2010).
- 4 Saga, G. et al. Embelin binds to human neuroserpin and impairs its polymerisation. *Sci Rep* 6, 18769, doi:10.1038/srep18769 (2016).
- 5 Santangelo, M. G. et al. On the molecular structure of human neuroserpin polymers. *Proteins* 80, 8-13, doi:10.1002/prot.23197 (2012).

3. AL amyloidosis (2017 - 2025)

Light chain amyloidosis (AL) is the most common systemic amyloidosis with an incidence of 10 new patients per million per year in western countries. It is a sporadic disease which strongly correlate with age. The two categories of patients most likely to develop AL are the ones presenting a monoclonal gammopathy of undetermined significance (MGUS) or the ones affected by multiple myeloma. AL is a very debilitating disease, and the frequent cardiac involvement is often life threatening.

My group have been focusing on several aspects of this highly complex disease: the biophysical properties of amyloidogenic light chain (LC) dimers, the specific molecular traits underlying the cardiac toxicity observed in patients and the design of inhibitors of such toxicity. Moreover, the structural and biochemical characterization of amyloid deposits extracted from patients and their interactome.

Main publications:

1. Oberti, et al. 2017. Concurrent structural and biophysical traits link with immunoglobulin light chains amyloid propensity. *Sci Rep*, 7(1): p. 16809.
2. Paissoni, et al. 2025. A conformational fingerprint for amyloidogenic light chains. *Elife*, 13.
3. Brogginini, et al. 2023. Nanobodies counteract the toxicity of an amyloidogenic light chain by stabilizing a partially open dimeric conformation. *J Mol Biol*, 435(24): p. 168320.
4. Maritan, et al. 2020. Inherent Biophysical Properties Modulate the Toxicity of Soluble Amyloidogenic Light Chains. *J Mol Biol*, 432(4): p. 845-860.
5. Lavatelli, et al. 2020. Mass spectrometry characterization of light chain fragmentation sites in cardiac AL amyloidosis: insights into the timing of proteolysis. *J Biol Chem*, 295(49): p. 16572-16584.
6. Mazzini, et al. 2021. Protease-sensitive regions in amyloid light chains: what a common pattern of fragmentation across organs suggests about aggregation. *FEBS J*.
7. Puri, et al. 2023. The Cryo-EM STRUCTURE of Renal Amyloid Fibril Suggests Structurally Homogeneous Multiorgan Aggregation in AL Amyloidosis. *J Mol Biol*, 435(18): p. 168215.

8. Schulte, et al. 2022. Cryo-EM structure of ex vivo fibrils associated with extreme AA amyloidosis prevalence in a cat shelter. *Nat Commun*, 13(1): p. 7041.
9. Schulte, et al. 2024. Helical superstructures between amyloid and collagen in cardiac fibrils from a patient with AL amyloidosis. *Nat Commun*, 15(1): p. 6359.
10. Swuec, et al. 2019. Cryo-EM structure of cardiac amyloid fibrils from an immunoglobulin light chain AL amyloidosis patient. *Nat Commun*, 10(1): p. 1269.

4. Beta-2 microglobulin (2007 – 2023)

Wild type beta-2 microglobulin (b2m) is the light chain of the Major Histocompatibility complex Class I synthesized by all nucleated cells in humans. B2m is known to be responsible for Dialysis Related Amyloidosis (DRA), a condition caused by accumulation of circulating b2m due to kidney failure. This high concentration of b2m leads to amyloid deposition in joints, bones and muscles.

Through mutational studies I have characterised: one of b2m regions more relevant for determining b2m aggregation propensity; the structural properties of b2m fibrils.

Main publications:

- 1 Barbet-Massin, E. et al. Rapid proton-detected NMR assignment for proteins with fast magic angle spinning. *Journal of the American Chemical Society* 136, 12489-12497 (2014).
- 2 Barbet-Massin, E. et al. Fibrillar vs crystalline full-length beta-2-microglobulin studied by high-resolution solid-state NMR spectroscopy. *Journal of the American Chemical Society* 132, 5556-5557 (2010).
- 3 Esposito, G. et al. The controlling roles of Trp60 and Trp95 in beta2-microglobulin function, folding and amyloid aggregation properties. *J Mol Biol* 378, 885-895 (2008).
- 4 Ricagno, S. et al. DE loop mutations affect beta-2 microglobulin stability and amyloid aggregation. *Biochem Biophys Res Commun* 377, 146-150 (2008).
- 5 Ricagno, S., Raimondi, S., Giorgetti, S., Bellotti, V. & Bolognesi, M. Human beta-2 microglobulin W60V mutant structure: Implications for stability and amyloid aggregation. *Biochem Biophys Res Commun* 380, 543-547 (2009).
- 6 Santambrogio, C. et al. DE-loop mutations affect beta2 microglobulin stability, oligomerization, and the low-pH unfolded form. *Protein Sci* 19, 1386-1394 (2010).

In 2012, I contributed to the identification of the first natural b2m mutant (D76N) responsible for a familial systemic amyloidosis unrelated to DRA, this work was published in *NEJM* (see below). Given the scientific interest of this mutant, I concentrated my efforts in clarifying the properties of the mutant protein in physiologic and pathologic settings, unveiling the molecular determinants of D76N aggregation propensity. Moreover in 2021 we reported a novel pathologic role for b2m amyloid aggregation: we showed that b2m aggregation in tumour associated macrophages (TAMs) of patients affected by Multiple Myeloma promotes inflammation leading to tumour survival and progression.

Main publications:

- 1 de Rosa, M. et al. Decoding the Structural Bases of D76N ss2-Microglobulin High Amyloidogenicity through Crystallography and Asn-Scan Mutagenesis. *PLoS One* 10, e0144061, doi:10.1371/journal.pone.0144061 (2015).
- 2 Halabelian, L. et al. Class I Major Histocompatibility Complex, the Trojan Horse for Secretion of Amyloidogenic beta2-Microglobulin. *J Biol Chem* 289, 3318-3327, doi: 10.1074/jbc.M113.524157 (2014).
- 3 Le Marchand, T. et al. Conformational dynamics in crystals reveal the molecular bases for D76N beta-2 microglobulin aggregation propensity. *Nature communications* 9, 1658, doi:10.1038/s41467-018-04078-y (2018).
- 4 Valleix, S. et al. Hereditary systemic amyloidosis due to Asp76Asn variant beta2-microglobulin. *N Engl J Med* 366, 2276-2283, doi:10.1056/NEJMoa1201356 (2012).
- 5 Hofbauer, D. et al. beta(2)-microglobulin triggers NLRP3 inflammasome activation in tumor-associated macrophages to promote multiple myeloma progression. *Immunity* 54, 1772-1787 e1779, doi:10.1016/j.immuni.2021.07.002 (2021).