Research activity of the Tor Vergata Biophysics group – PI: Silvia Morante

The scientific activity carried out in the Tor Vergata Biophysics group finds its place within the vast research area that is trying to tackle the challenging problem of understanding the physico-chemical basis of protein misfolding and aggregation as the latter processes are at the origin of plaque formation in severe amyloid related pathologies. Within the vast group of pathologies (more than 20) characterized by extra-cellular deposition of fibrillar material and generically called Amyloidosis, we have firstly focused our study on the Prion disease [Morante 2004; Guerrieri 2009; Minicozzi 2010; Stellato 2011; Stellato 2014] and then on the Alzheimer's Disease (AD) [Minicozzi 2008; Giannozzi 2012; Maiorana 2013; Morante 2014; De Santis 2015; Di Carlo 2015]. The latter is a progressive and devastating neurodegenerative disorder affecting an important fraction of the world aged population. AD accounts for 50% to 80% of dementia cases and is the sixth leading cause of death in developed countries. It causes severe problems with memory, thinking and behavior. Unfortunately, an effective therapy is still missing and the available drug treatments are only able to reduce the symptoms.
Well-identified peptides, the so-called Aβ-peptides originating from the cleavage of a membrane protein, the Amyloid Precursor Protein - APP, undergo a misfolding and aggregation process that leads to plaques formation, the hallmark of the AD development. An important, but not yet fully elucidated, role appears to be played in these processes by transition metals (mainly copper and zinc) that have been observed to be present in fairly large amount in patient's neurological plaques.
In recent years we have worked out specific experimental and theoretical techniques aimed at studying the structural properties of amyloid formation and on the effect of metals and small molecules on aggregation and fibrillation processes.

E. De Santis, V. Minicozzi, O., Proux, G.C. Rossi, K.I., Silva, M.J. Lawless, … and S. Morante (2015) *The Journal of Physical Chemistry B*, **119(52):** 15813-15820.

M.G. Di Carlo, V. Minicozzi, V. Foderà, V. Militello, V. Vetri, S. Morante, and M. Leone (2015) *Biophysical chemistry*, *206*, 1-11.

P. Giannozzi, K. Jansen, G. La Penna, V. Minicozzi, S. Morante, G.C.. Rossi, and F. Stellato, F. (2012) *Metallomics*, **4(2)**: 156-165.

F. Guerrieri, V. Minicozzi, S. Morante, G.C. Rossi, S. Furlan, and G. La Penna (2009) *JBIC Journal of Biological Inorganic Chemistry*, **14(3):** 361-374.

A. Maiorana, T. Marino, V. Minicozzi, S. Morante, S. and N. Russo (2013). *Biophysical chemistry*, **182**: 86-93.

V. Minicozzi and S. Morante, S. *International Journal of Quantum Chemistry*, **110(3)**: 656-680.

V. Minicozzi, F. Stellato, M. Comai, M. Dalla Serra, C. Potrich, W. Meyer-Klaucke and S. Morante (2008) *Journal of Biological Chemistry*, **283(16):** 10784-10792.

S. Morante, R. González-Iglesias, C. Potrich, C. Meneghini, W. Meyer-Klaucke, G. Menestrina, and M. Gasset (2004) *Journal of Biological Chemistry*, **279(12):** 11753-11759.

S. Morante, and G.C. Rossi (2014) *Advanc Alz Res*, **2:** 100-147.

F. Stellato, A. Spevacek, O. Proux, V. Minicozzi, G.L. Millhauser, G. and S. Morante (2011) *European biophysics journal*, **40(11)**: 1259-1270.

F. Stellato, V. Minicozzi, G.L., Millhauser, M. Pascucci, O. Proux, G.C. Rossi, ... and S. Morante *European Biophysics Journal*, **43(12):** 631-642.

Report of the 2018 Scientific Activity

1- Protein aggregation in neurodegenerative diseases (on going)

Since more than fifteen years we have been working on understanding protein aggregation in the presence and in the absence of metal ions both experimentally by using X-ray Absorption Spectroscopy (XAS) and theoretically with the help of multi-level classical and *ab initio* simulations.
We have used classical molecular dynamics to study systems composed by hundred thousand atoms over the microsecond time-scale. This approach is particularly useful when the interest is in studying the interaction among various kinds of biological macromolecules and/or in following their aggregation process. On the experimental side, we performed XAS measurements to study Aβ aggregation (and its possible modulation) in the simultaneous presence of metal ions and β-sheet breaker peptides.
We are currently taking advantage of coarse grained theoretical models to study the interaction of full fibrils with small molecules, called β-sheet breaker peptides, that we proved to be able to delay Aβ peptide fibril formation [Minicozzi 2014, Stellato 2017].

V. Minicozzi, R. Chiaraluce, V. Consalvi, C. Giordano, C. Narcisi, P. Punzi, … and S. Morante (2014) *Journal of Biological Chemistry*, **289(16)**: 11242-11252.

F. Stellato, Z., Fusco, R. Chiaraluce, V. Consalvi, S. Dinarelli, E. Placidi, ... and S. Morante (2017) *Biophysical chemistry*, **229:** 110-114.

2- Protein aggregation in diabetes

It has been observed that the interaction of the pro-peptide ProIAPP1–48 with metal ions has an impact on the diabetes cytotoxicity of the peptides as well as on their deposition in the form of amyloid fibrils. In particular, Cu(II) seems to inhibit amyloid fibril formation, thus suggesting that Cu homeostasis imbalance may be involved in the pathogenesis of type 2 diabetes mellitus. We performed X-ray Absorption Spectroscopy (XAS) measurements of Cu(II)-ProIAPP complexes at near-physiological, 10 μM, equimolar concentration of Cu(II) and peptide. X-ray experiments at such a low concentration have been made possible thanks to the use of the High Energy Resolved Fluorescence Detection XAS facility recently installed at the ESRF beamline BM16 (FAME-UHD). Our preliminary data [De Santis 2019] show that XAS measurements at micromolar concentrations are feasible and confirm that ProIAPP1–48-Cu(II) binding at near-physiological conditions can be detected.

E. De Santis, E. Shardlow, F. Stellato, O. Proux, G. Rossi, C. Exley and S. Morante (2019) *Condens Matter.* **4(1)**:13.

3. Multi-scale theoretical approach to XAS: the case of Zn(II) in water

We studied the Zn(II) coordination mode in water by performing a *first principle* computation of the low energy X-ray absorption spectrum region based on a density functional theory (DFT) calculation of the electronic potential [Stellato 2018]. Classical molecular dynamics (MD) simulations and the dummy atoms method are used to build the initial geometrical configuration simulated system.
Many DFT relaxed structures have been used for the *first principle* computation of the low energy part of the X-ray absorption spectrum. The comparison of the average with experimental data shows that the Zn in water lives in an octahedral coordination.

F. Stellato, M. Calandra, F. D'Acapito, E. De Santis, G. La Penna, G. Rossi, S. Morante (2018) *Physical Chemistry Chemical Physics* **38**: 24775-24782.

4. Interaction between D2 cochlear receptor and ototoxic molecules (on going)

The medical problem of interest is to determine whether environmental factors are responsible for enhancing Sensor Neural Hearing Loss (SNHL). SNHL has been proved to speed up the development of

neurodegenerative diseases like Parkinson or Alzheimer diseases. We are planning to use a molecular docking procedure and free-energy calculations to find the (best) interaction sites of ototoxic molecules in the D2 cochlear receptor (this project has been partially funded by INAIL (Project BRiC 2016 ID17/2016).

5. Anti-cancer peptides

Peptides bound to radioactive elements are used in oncology to target cancer cells thanks to their selectivity, with the consequence that, because of this very selectivity, one needs a specific radio-peptide for each tumour type. To overcome this problem, we designed a functionalized peptide capable of switching its net charge in a pH-dependent way and specifically binding to tumour membranes as they are negatively charged in a large number of tumours. We used classical molecular dynamics to study the interaction between the peptide we designed with models of tumour and healthy human cell membranes [Capozzi 2018]. We showed that the peptide has a significantly larger affinity to tumour membranes than to healthy tissues, thus suggesting that it can be a promising theranostic tool in cancer diagnostic and therapy. To validate our simulations, we are planning to perform in vivo experiments on animal models and we are currently in touch with researchers of the Faculty of Medicine and Surgery.

E. Capozzi, V. Minicozzi, S. Aureli, G.C. Rossi, F. Stellato, and S. Morante (2018) *BBA Biomembranes* **1860(11)**: 2348-2355.

6. MD simulations and fluorescence experiments of the stability of a TNF receptor-associated factor trimer.(V. Minicozzi)

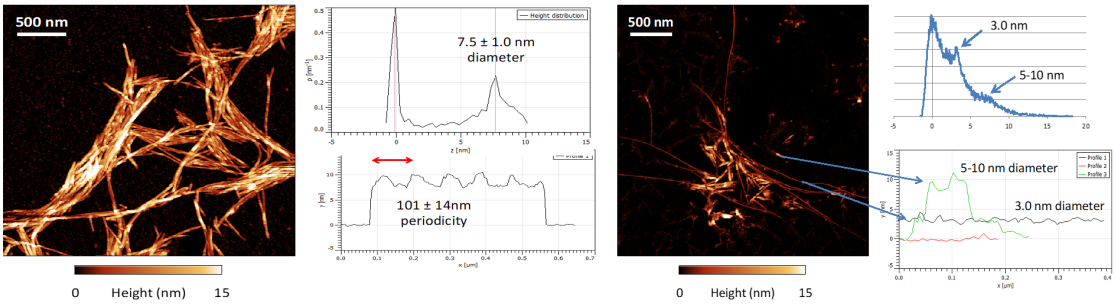
TNF receptor-associated factors (TRAFs), at different pressures and pH values in order to find out the regions of the protein that trigger the trimer dissociation with increasing pressure and/or decreasing pH. Moreover, we studied by several different experimental techniques and MD simulations the effect of single aminoacid change on the secondary structure and on the flexibility of Frataxin, a protein connected to Friedrich's ataxia.

New Projects

1. Structural biology experiments at FELs

FELs opened up the way for an unprecedented wide class of experiments in structural biology [Ferrario 2018]. Thanks to the high peak brilliance and to the short duration of the FEL pulses, measurements in the so-called diffract-and-destroy regime can be performed thus overcoming radiation damage, which is one of the main limitations of synchrotron radiation based experiments. The idea of diffract-and-destroy experiments had been proposed in the early 2000s when classical molecular dynamics simulations confirmed that, exploiting short FEL pulses, useful structural information could be collected before the sample was completely destroyed by the interaction with X-rays. The advantage of performing this kind of experiments at FELs rather than at a synchrotron is twofold: first of all, as mentioned above the very high peak brilliance of the FEL pulses allows acquiring, data so fast that the samples appear not to be altered by radiation damage. Second, thanks to the short duration of the pulses, time-resolved experiments with high temporal resolution are possible. This means that conformational changes may be observed at a time-resolution ranging from femtoseconds to microseconds by having a pump pulse exciting the samples and then a probe FEL pulse coming with varying time delays acquiring data of the samples in the excited state.
We just submitted a proposal at the European X-FEL to perform fiber diffraction measurements on

different α-syn aggregates and fibrils, that are known to be involved in Parkinson's disease (PD) development. PD is the second most common neurodegenerative disease after Alzheimer's disease and the most common movement disorder, affecting nearly 3% of the population over age 70.



AFM morphometric analysis of α-syn fibrils, α-syn WT (left) and α-syn (1-99) (right), height distributions (upper right columns) and fibril periodicity measured by profile analysis (lower right columns)

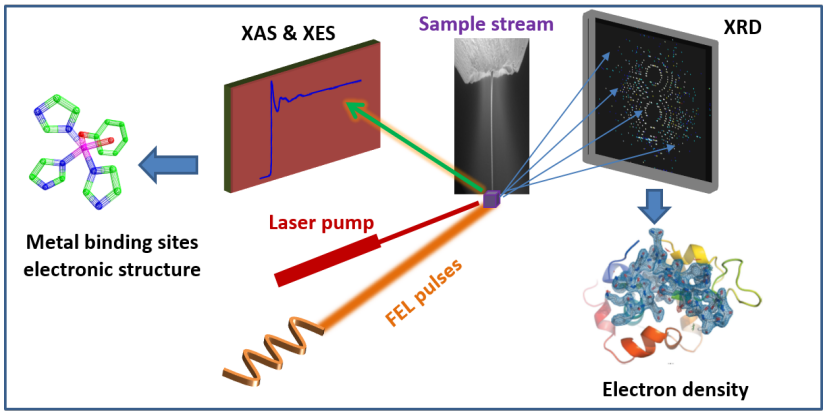
M. Ferrario, …., S. Morante et al., (2018) *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **909**: 134-138.

2. Computational approaches to assess the impact of amino acid variations on protein structure, function and protein-protein binding affinity.

We have been funded by the PRIN-2018 (local coordinator G. Salina). Within the PRIN project the main aim of our Unit will consist in filling the gap between thermodynamic and disease-related information on protein variants. We propose, for the first time, to integrate theoretical/computational approaches with experimental validations to assess the impact of amino acid variations on protein structure, function and protein-protein binding affinity.

3. Structural biology experiments @ MariX

The MariX FEL will provide high intensity pulses, with up to 10^{10} photon/pulse, in the X-ray region [Serafini, 2019]. MariX FEL delivers a relatively low number of photons/pulse if compared to other FEL sources, but it can work with at a quite higher repetition rate (of the order of a MHz) with a few femtosecond pulse length. These features can be exploited for applications to a number of problems of biological relevance. Furthermore, the MariX FEL facility will allow to successfully perform serial X-ray crystallography measurements. As illustrated in Figure 1, the two types of experiments can be simultaneously performed.



A schematic apparatus aiming at (simultaneously) performing XRD and XAS/XES measurements at the MariX FEL.

L. Serafini, …, S. Morante et al., (2019) *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **930:** 167-172.

4. Amyloid fibrils unravelled by neutron scattering & molecular dynamics simulations

We submitted a project to the "Beyond Borders 2019" call of the Tor Vergata University. The goal of the project is to observe and classify the various types of aggregates (amorphous and fibrillar) formed by proteins that undergo misfolding and aggregation. More specifically, the project will be mainly focused on the amyloid beta (Aβ) peptides, whose misfolding and aggregation is known to be involved in the pathogenesis of Alzheimer's diseases. The purpose of this study is to identify structural differences of the Aβ peptide aggregates possibly due to different conditions of the surrounding environment. We will use Molecular Dynamics simulations to interpret Small Angle Neutron Scattering (SANS) experimental measurements.

5. Naringenin interaction with biological membranes (V.Minicozzi)

The research will be carried out by MD simulations which will be able to give us information about the ability of Naringenin to insert into the bilayer, the insertion dependence on Naringenin concentration and the changes of bilayer properties due to Naringenin presence

6. Anti-tumor carriers (V.Minicozzi)

We will perform MD study of the interaction between short peptides and specific receptors located on the blood brain barriers (BBB).

International and National collaborations:

- Prof. F. Buda and dr. A. Sevink – Leiden University, The Netherlands
- Prof. M. Calandra - Directeur de Recherche CNRS Institut des Nanosciences de Paris Université Pierre et Marie Curie, France
- Prof. C. Exley – Keele University, UK
- Dr. M. Ferrario – LNF/INFN Frascati - Italy
- Dr. O. Proux – Observatoire des Sciences de l'Université de Grenoble, France
- Prof. V. Consalvi and Prof. R. Chiaraluce – Sapienza University, Rome, Italy
- Dr. G. La Penna – CNR, Firenze, Italy
- Prof O. Schillaci – Facoltà di Medicina e Chirurgia - Tor Vergata - Roma, Italy
- Prof. F. Spinozzi- Dip. Scienze della Vita e dell'Ambiente Università Politecnica delle Marche, Ancona, Italy