REPORT –3rd Ph.D. Year

Davide Odino (XXXV Cycle)

Supervisors: Prof.ssa Annalisa Relini, Prof. Claudio Canale

RESEARCH ACTIVITY

My PhD project focuses on applications of fluorescence microscopy and scanning probe microscopy to the structural and mechanical characterization of biological samples and interfaces for the study of protein aggregation diseases, including the effects of drugs against aggregation.

During the third year of my PhD, I studied the formation of amyloid aggregates. The latter are highly organized and insoluble fibrillar aggregates that are formed due to misfolding and aggregation of soluble proteins due to destabilization of their native structure. The ability to form amyloid assemblies is a generic property of polypeptide chains. In fact, regardless of the different amino acid sequences of the proteins, the aggregates formed have a typical fibrillar structure with high β sheet content. An entire class of pathologies (including Alzheimer's disease and Parkinson's disease) is associated with the deposition of intracellular or extracellular misfolded protein aggregates [1]. Therefore, understanding the molecular mechanism of protein aggregation is a crucial issue to counteract misfolded protein diseases. To this purpose, fluorescence microscopy (confocal microscopy and stimulated emission depletion (STED) microscopy) and a combined AFM-STED system can give a relevant contribution.

I investigated the in vitro aggregation process of a nonpathological protein, insulin from bovine pancreas. Insulin was fluorescently labelled either with ATTO 647-N (insulin-647) or ATTO 594 (insulin-594), both functionalized with an NHS ester group. We used a total labelled/unlabelled protein ratio of 1:20 (the ratio being 1:40 for each dye). We aimed to determine whether insulin-647 and insulin-594 were recruited together in the protein aggregates. From the analysis of the confocal and STED images, we found that many fibrils accepted a single fluorescent species, suggesting that a fraction of labeled peptides follow only selected aggregation pathways (Fig. 1). To confirm these results, fluorescence lifetime microscopy (FLIM) measurements are in progress. Furthermore, we used the correlative STED-AFM technique on the same samples to verify whether a fraction of unlabelled fibrils was present. This phenomenon had already been shown in the aggregation of insulin labeled with a single peptide, ATTO 488-NHS [2]. By analyzing the overlay of the STED image with the AFM topography, we obtain an average correlation coefficient [3] of 0.86 ± 0.04 (which represents the fraction of AFM that overlaps the STED image), showing that the great majority of the fibers are fluorescent (Fig. 2). However, the fluorescence intensity is very low in several fibrils, so fibrils are not homogeneously labeled; as confirmed by the mean Mander's threshold coefficient [3] equal to 0.59 ± 0.04.

In the context of the characterization of amyloid aggregates with AFM-fluorescence microscopy, I also tested the efficiency of pFTAA (the pentameric form of formyl thiophene acetic acid) in the recognition of Abeta

fibers (Fig. 3). The work has been done in collaboration with the group of Prof. Maria Sunnerhagen of the Department of Physics, Chemistry and Biology of the University of Linköping (Sweden). This group works on developing fluorophores with a strong affinity for beta-sheet; such fluorophores can be used to mark the structure of amyloid aggregates, similarly to thioflavin T. This collaboration is part of the Mosbri (Molecular-Scale Biophysics Research Infrastructure) European Project.

In the second half of the second year, always within the study of the pathological mechanisms involving amyloidogenic proteins, I studied the effect of alpha-synuclein, the protein involved in Parkinson's disease, on the mechanical properties of microtubules. The latter are components of the cytoskeleton, which is involved in several cellular activities and provides shape and stiffness to the cell [4]. Microtubules result from the polymerization of tubulin protein, forming a hollow tube about 25 nm in diameter. In this study, which started last year, I analyzed the mechanical properties and morphological characteristics of microtubules polymerized in the absence and the presence of two alloforms of alpha-synuclein: a non-pathological form (wild type) and a pathological form (A53T mutant) at a molar ratio tubulin:alpha-synuclein of 1:8.

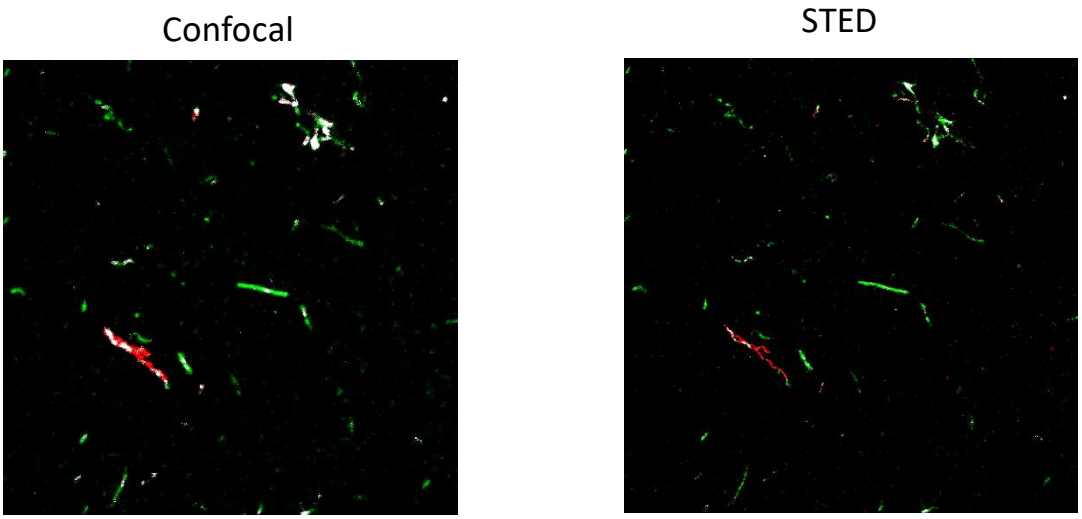

Figure 1. Insulin fibrils at a dye-to-protein ratio of 1:20. In the two figures, the fibers marked only with ATTO 594 appear green, the fibers marked exclusively with ATTO 647 appear red, the fibers with both markers are shown in white. Image size: 27 µm.

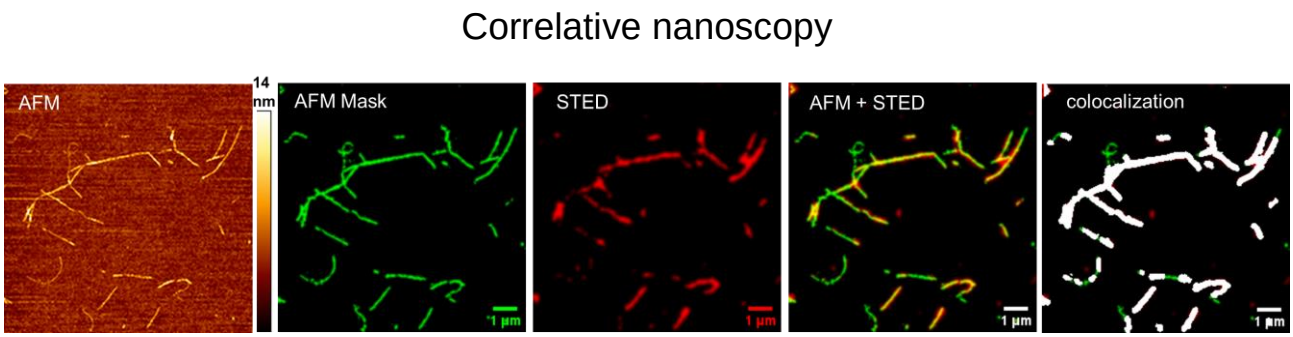

Figure 2. Insulin fibrils at a dye-to-protein ratio of 1:20. Image size: 8 µm; the Z range in the AFM image is 14 nm.

Correlative nanoscopy

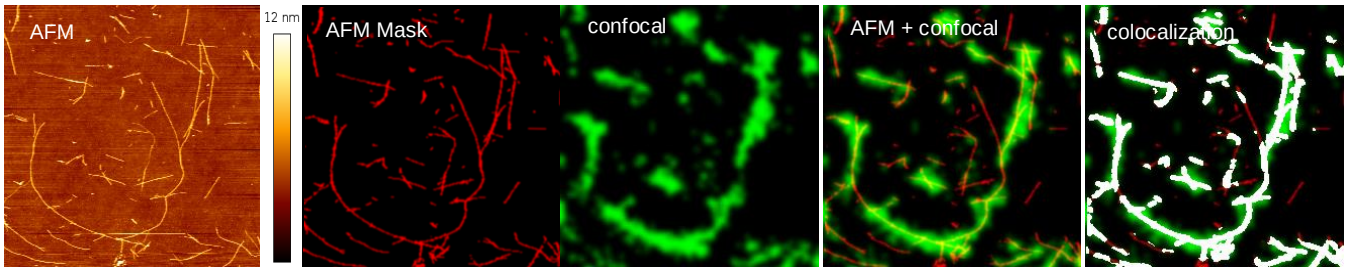

Figure 3. Aβ fibrils labeled with pFTAA. Image size: 6 µm; the Z range in the AFM image is 12 nm.

[1] C. M. Dobson. The structural basis of protein folding and its links with human disease. *Phil. Trans. R. Soc. Lond. B.* (2001) 356:133-145.

[2] M. Cosentino, C. Canale, P. Bianchini, A. Diaspro. AFM-STED correlative nanoscopy reveals a dark side in fluorescence microscopy imaging. Sci. Adv. (2019) 5: eaav8062.

[3] S. Bolte F. P. Cordelières. A guided tour into subcellular colocalization analysis in light microscopy. The Royal Microscopical Society, Journal of Microscopy. (2006) 224, 213–232.

[4] Iwan A. T. Schaap et al. Elastic Response, Buckling, and Instability of Microtubules under Radial Indentation. Biophysical Journal, Volume 91. (August 2006) 1521–1531.

EXAMS

Fisica applicata alla biomedicina e ai biomateriali. Prof Claudio Canale. Exam passed

CONFERENCES AND SCHOOLS

Poster: 1st MOSBRI Scientific Conference, Institut Pasteur, Paris – 20th-22nd June 2022. D. Odino*1, E. Angeli1, P. Bianchini2, E. Gatta1, V. Bazzurro1, A. Relini1, A. Diaspro1-2, C. Canale1 *A nanoscopic view on the aggregation process of partially labelled peptides solutions.* With bursary.

Flash talk: International School of Physics "Enrico Fermi" Varenna (LC); course: Multimodal and Nanoscale Optical Microscopy. *Amyloid fibrils formation observed with two-colors STED microscopy.*