Molecolar Biophysics
Department of Physics, University of Genoa, Italy

Ranieri Rolandi

Group present composition and activities

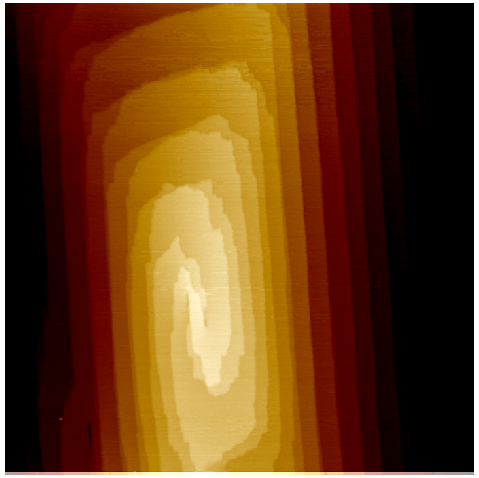
Permanent positions:	Post-docs	PhD student
Ornella Cavalleri, Annalisa Relini Ranieri Rolandi	Amanda Penco Daniela Nichino	Maurizio Bergamino

Protein amyloid aggregation
Protein-surface interaction

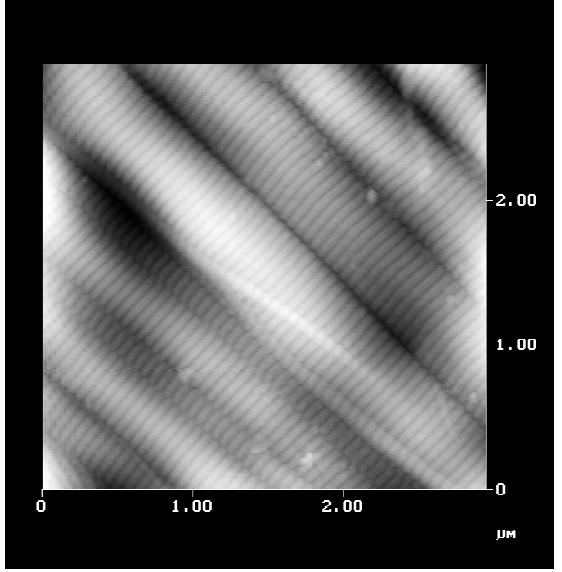
SPM (AFM), UV-visible spectroscopy, light scattering , X-ray spectroscopy, X-ray reflectivity, neutron scattering.

Past activities relevant to this COST Action

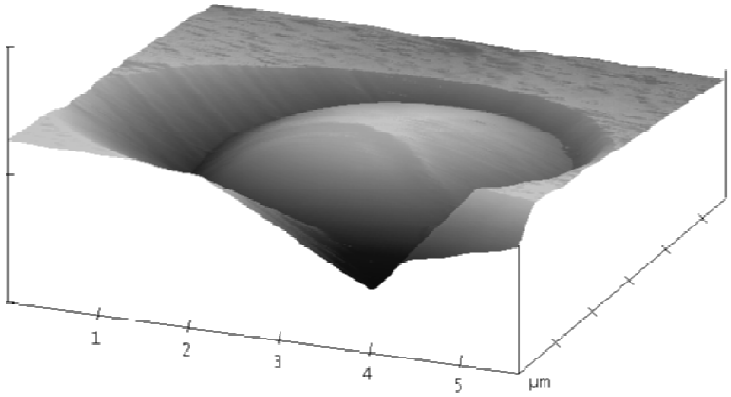
Collagen AFM imaging
Protein crystal AFM imaging
Myelin Basic Protein in model membranes
Force measurements on cells



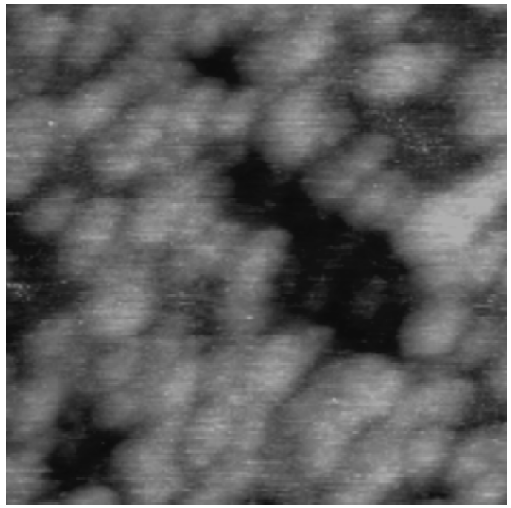
Relini A. et al., Langmuir, 19 (2003), 2908



Odetti P. et al., Diabetes /Metabolism Research and Reviews 16 (2000), 74



Svaldo Lanero T. et al. J. Biotechnol. 124 (2006), 723



Rispoli P. et al., Biophys. J. **93** (2007), 1999

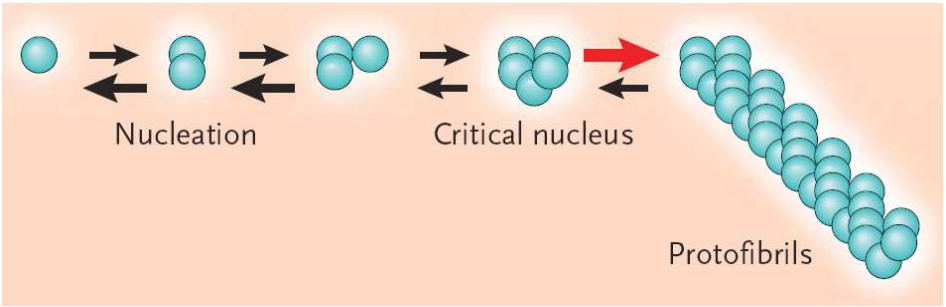
Amyloid aggregates and their interaction with lipid membranes

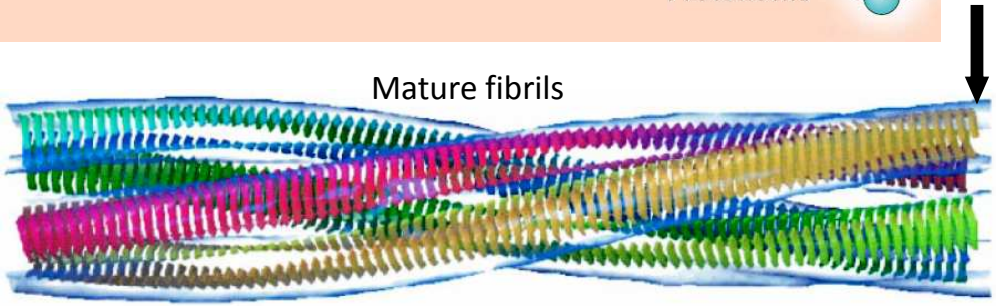
- Protein aggregation into amyloid fibrils or intracellular inclusions is involved in a number of severe pathological conditions (Alzheimer's disease, Parkinson's disease, spongiform encephalopathies…)

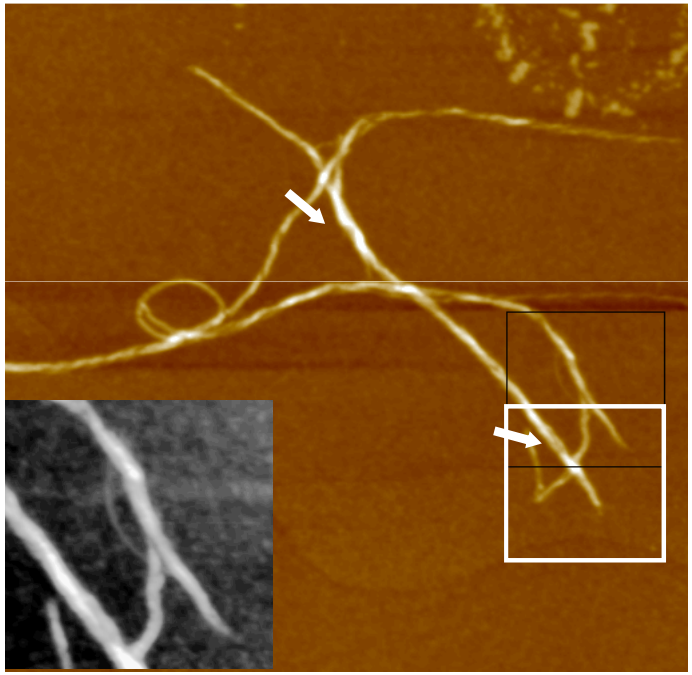
- Proteins unrelated to disease can form amyloid fibrils in vitro, suggesting that the ability to form amyloid fibrils is a generic property of polypeptide chains

- Amyloid fibrils represent a potentially interesting nanomaterial

Protein aggregation into amyloid fibrils





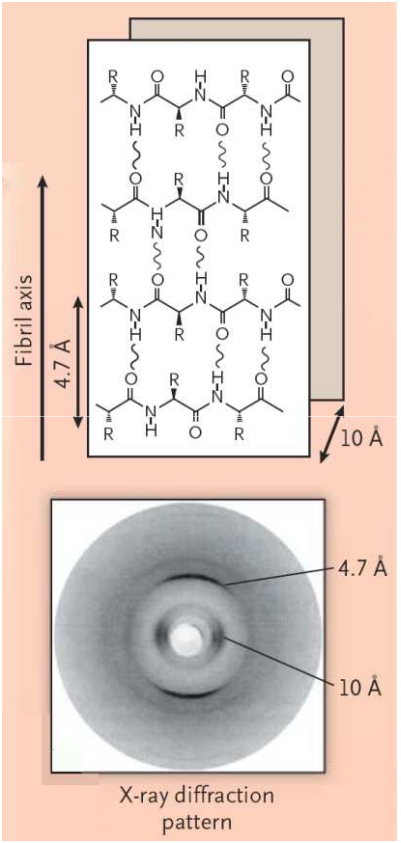


Scan size 2.8 µm, (inset 0.58 µm); Z range 40 nm.
A. Relini et al., (2004)
J. Mol. Biol. 338, 943-957.

F. Chiti and C. M. Dobson (2006) Annu. Rev. Biochem. 75:333–366.

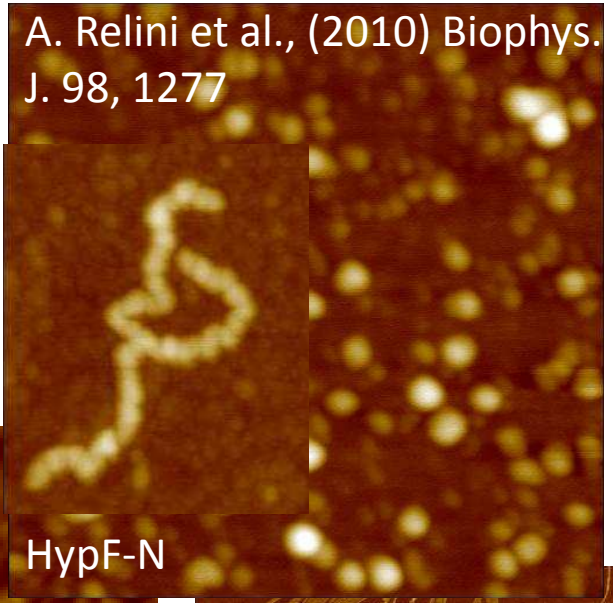
M. Stefani (2004) Biochim. Biophys. Acta 1739:5–25.

G. Merlini and V. Bellotti (2003) N. Engl. J. Med. 349:583-596.

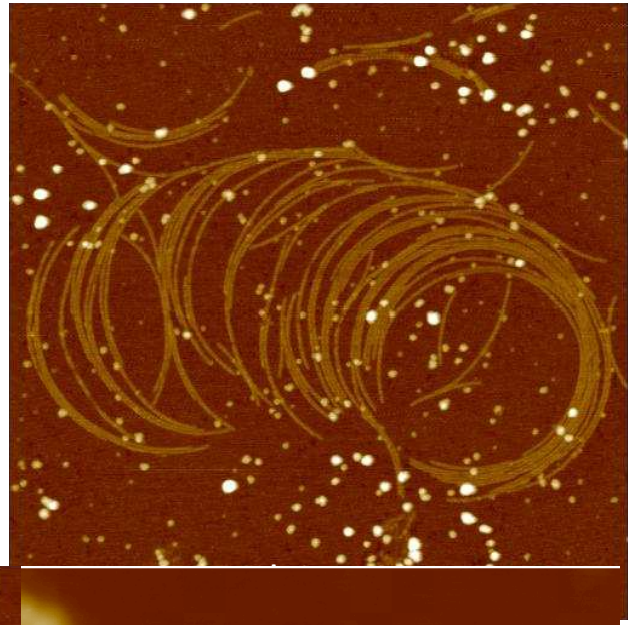


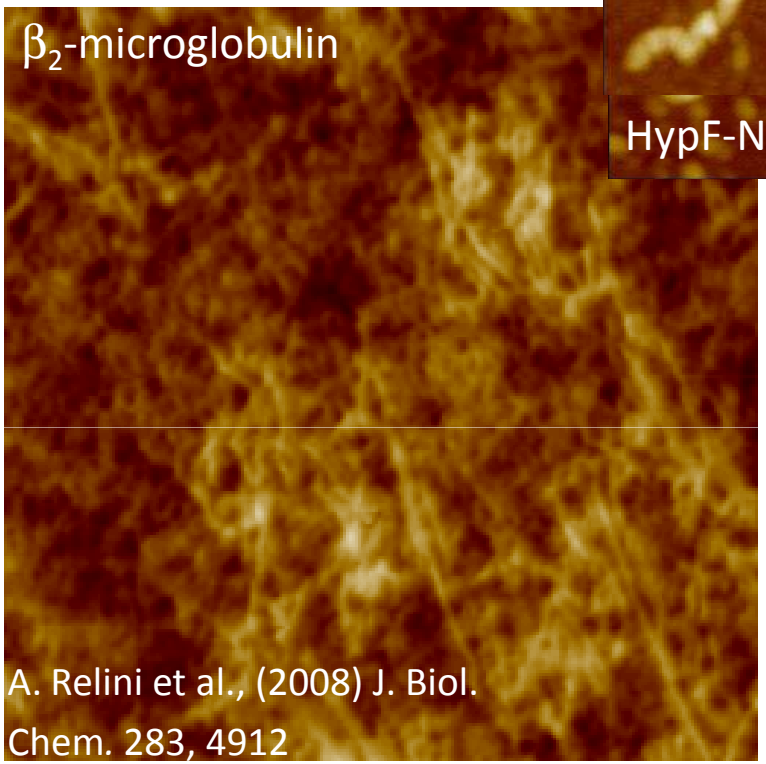
Amyloid aggregation can proceed through a variety of prefibrillar structures

Scan size 500 nm, Z range 8 nm

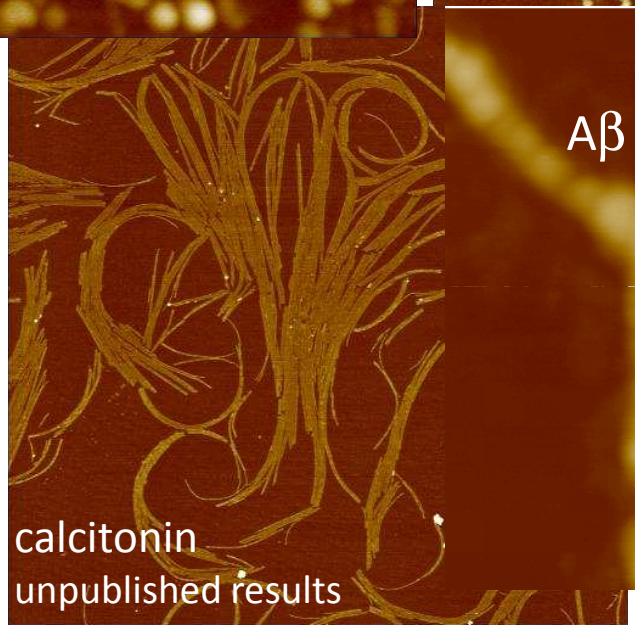


Scan size 2.1 µm, Z range 5 nm

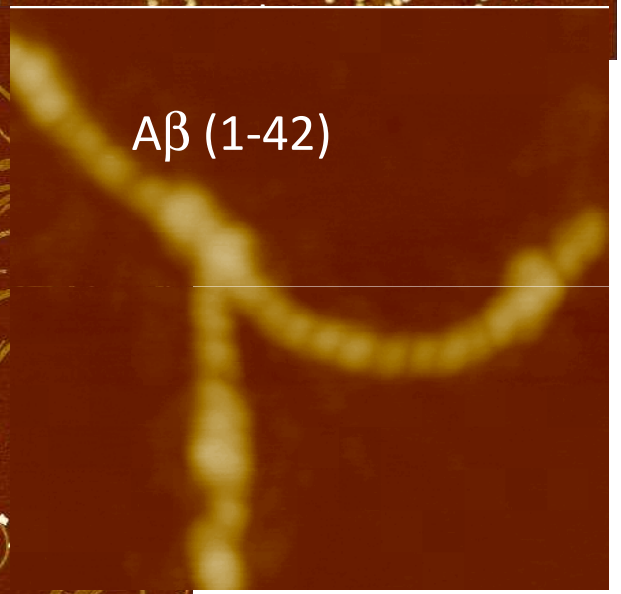




Scan size 600 nm, Z range 8 nm

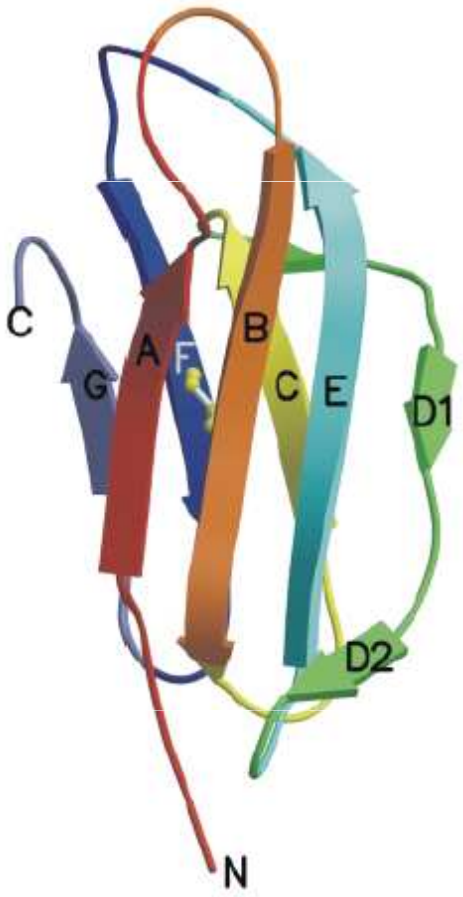


Scan size 5.4 µm, Z range 6 nm



Scan size 330 nm, Z range 13 nm

β_2-microglobulin



From Jones et al., J. Mol. Biol. 325 (2003), 249.

β_2-microglobulin is the light chain of the major histocompatibility complex class I

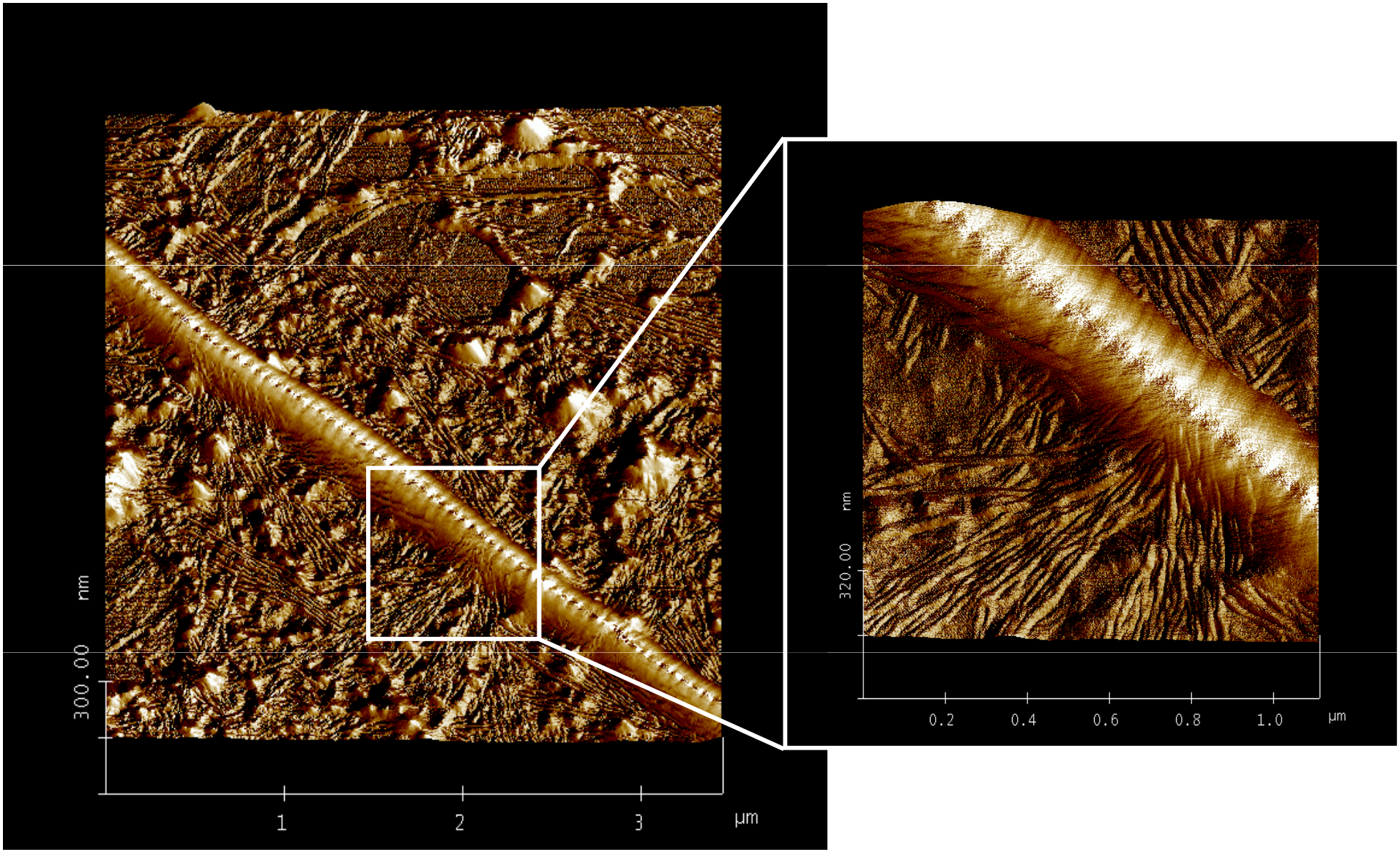
It is involved in dialysis-related amyloidosis

Amyloid fibrils are deposited in bones and ligaments

β_2-microglobulin alone does not form amyloid fibrils in vitro under physiologic conditions

Which factors determine its aggregation in vivo?

Analysis of *ex-vivo* fibrillar material



Amyloid fibrils are associated to collagen fibers

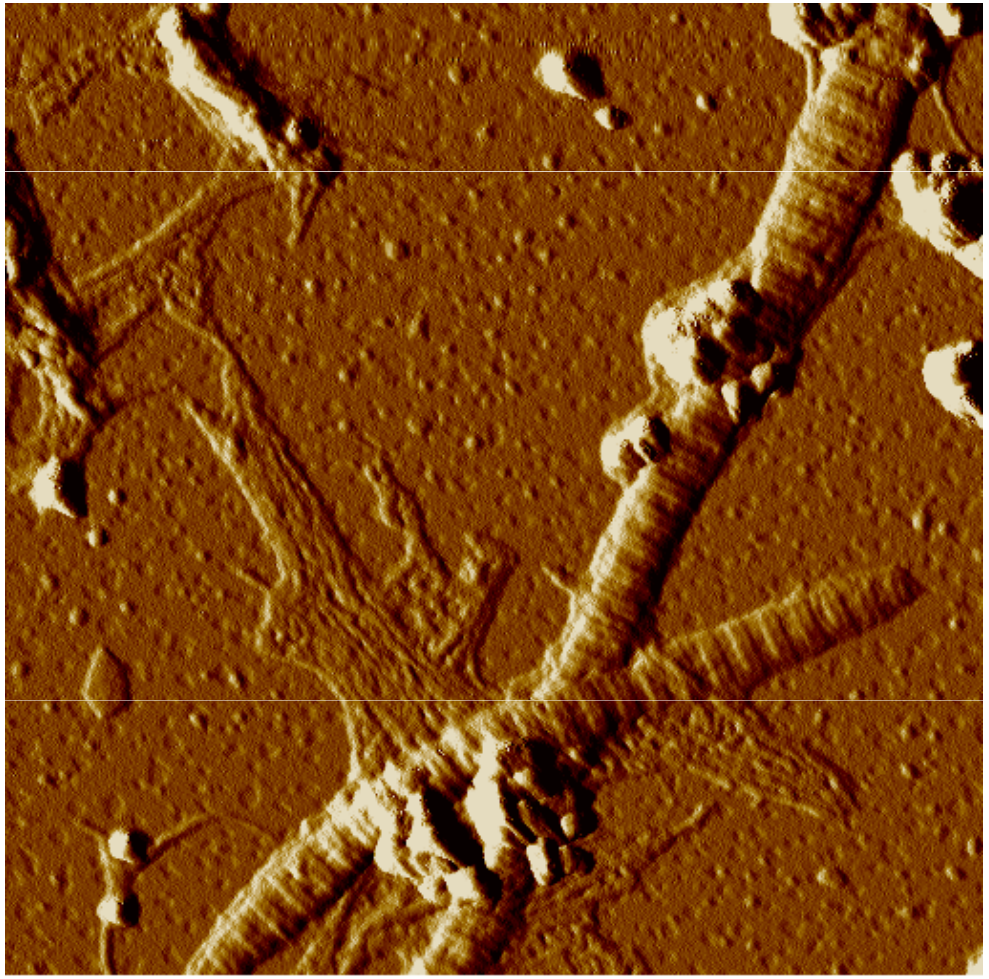
A. Relini et al. (2006) J. Biol. Chem. 281, 16521-16529.

Testing the effect of cofactors on the aggregation process

β_2-m fibrillation is accelerated by heparin

Fibrils are formed already after 24 h of aggregation

A. Relini et al. (2008) J. Biol. Chem. 283, 4912-4920.



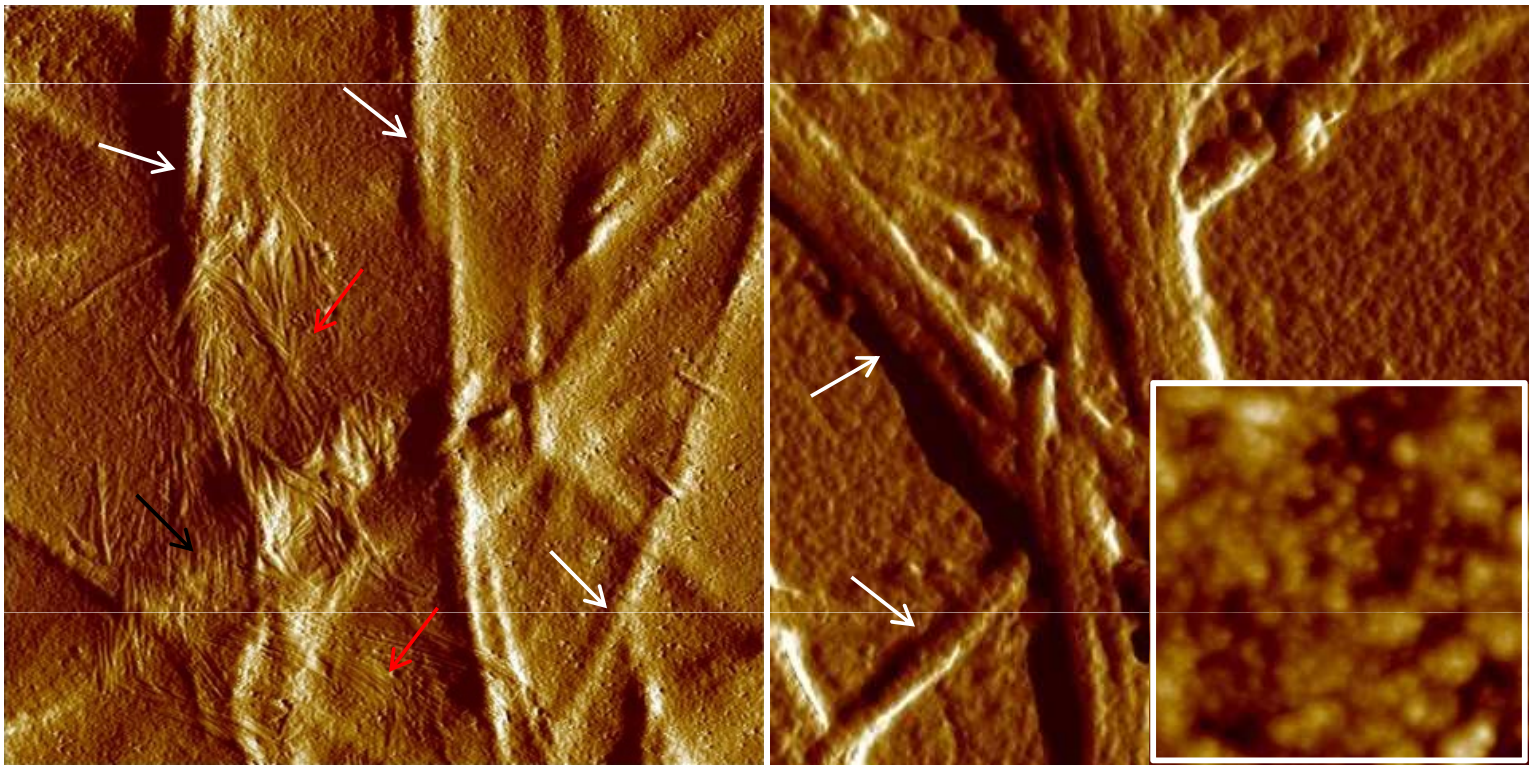
Amplitude data, scan size 2.0 µm

Testing drugs to inhibit aggregation

Doxycycline inhibits β_2-m fibril growth in the presence of collagen and heparin

without doxycycline

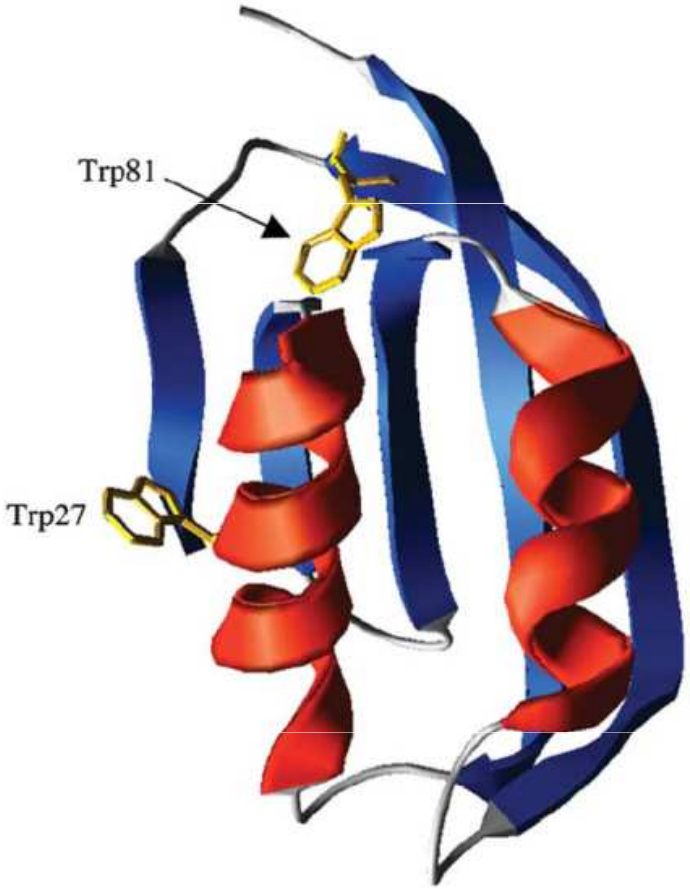
with doxycycline



Amplitude data, scan size 2.8 μ. Inset, height data, scan size 460 nm, Z range 20 nm. White arrows, collagen fibers; red arrows, amyloid fibrils

Giorgetti S et al. J. Biol. Chem. 2011; 286:2121-2131, Paper of the Week

Model protein: HypF-N

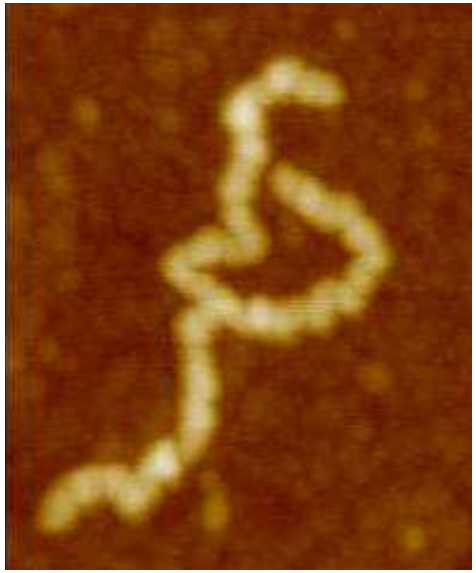


The , 91-residue, 10452 Da, **N-terminal domain of *E. coli* hydrogenase maturation factor (HypF-N)** is a stably folded α/β protein with a ferredoxin-like fold

C. Rosano et al. (2002), J. Mol. Biol. 321, 785-796.

This protein undergoes amyloid aggregation in the presence of trifluoroethanol (TFE) and at low pHs and its pre fibrillar aggregates are toxic to cultured cells. It represents a useful model to study fibrillogenesis since the different aggregates are stable when transferred to conditions different from those that promote their formation.

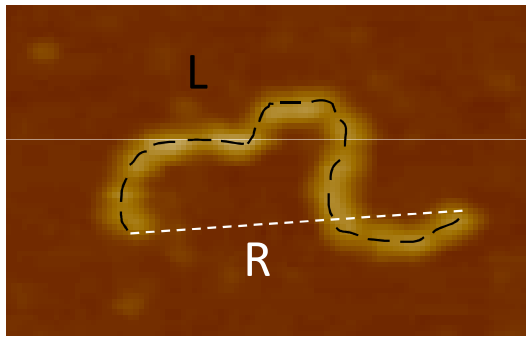
Protofibrils



Low pHs and high ionic strength

- HypF-N at low pHs and high ionic strength forms worm like protofibrils formed by roughly sferical oligomers.

- The analysis of **the end-to-end distance (R)**, distribution in relation to the **contour length (L)** distribution shows the existence of **two population with different contour lengths.**



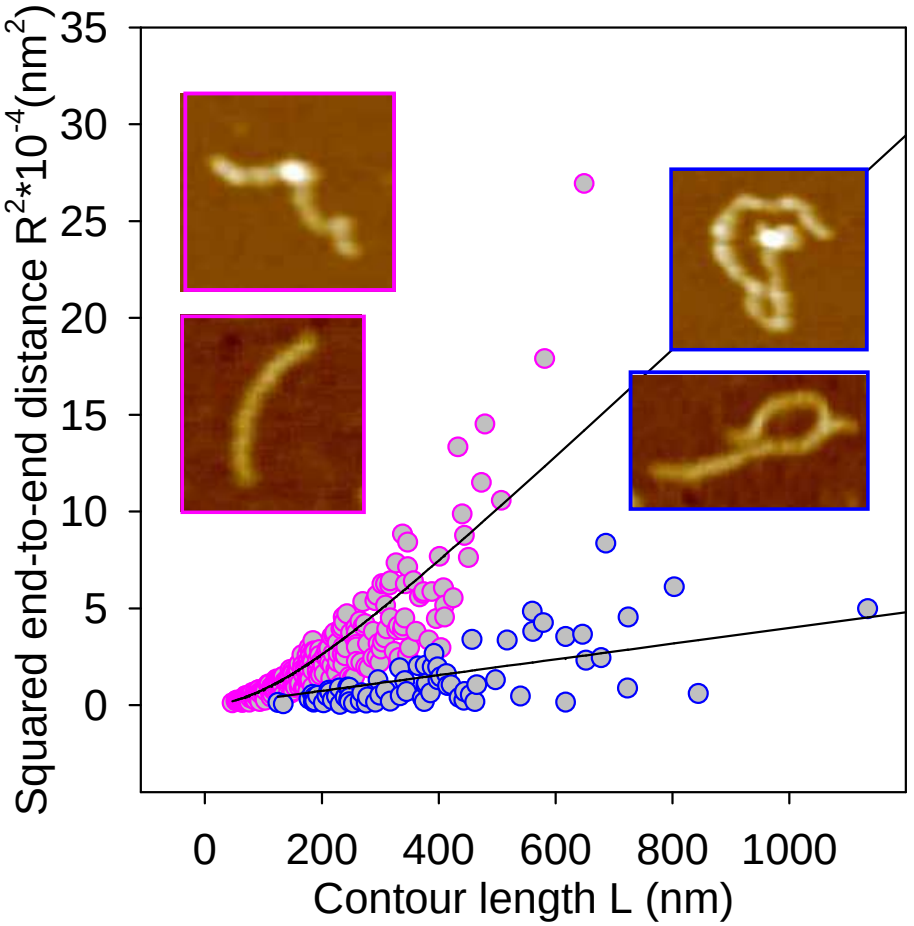
A. Relini et. al. (2010) Biophys. J., 98: 1277

To discriminate the two population we considered the value η^* corresponding to the minimum of the distribution and we separated the data with $\eta < \eta^*$ from those with $\eta > \eta^*$

Squared end-to-end distance (R^2) versus contour length L of protofibrils of the population 1 ($\eta < \eta^*$) and the population 2 ($\eta > \eta^*$). The lines are the predicted values obtained fitting the equation:

$$\left\langle R^2 \right\rangle_{2D} = 4PL\left(1 - \frac{2P}{L}\left(1 - e^{-\frac{L}{2P}}\right)\right)$$

to the experimental data.



P(1) = 69 ± 3 nm; P(2) = 10 ± 1 nm

•Structural degeneracy of prefibrillar aggregates was previously observed in mature fibrils [T. P. Knowles et al. (2006) PRL, 96:23831; A. T. Petkova et al. (2005) Science, 307:262]

•The differences of the physical properties of the two populations suggest that they may have different effects and different toxicity on cells.

Differences in packing and exposure to solvent of hydrophobic residues determine the interactions with cell membranes and cytotoxic effects of different Hypf-N oligomers. *S. Campioni et al., 2010, Nature Chem. Biol.* ***6****, 140-147*

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Résumé

The group is involved in research projects on amyloid aggregates and on interaction of biological molecules with inorganic surfaces

What we can do for Cost Action TD 1002

Share our expertise on AFM imaging of proteins, membranes, cells.
Actively take part in a bio-med AFM network

What we expect from Cost Action TD 1002

Ideas, stimulus, new collaborations, an active bio-med AFM network

Acknowledgments

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