Research Highlight #201

Francesco Simone Ruggeri, Ph.D.

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Professor Francesco Simone Ruggeri characterizes biomolecular processes, polymers, and (bio)materials at the nanoscale. He has contributed significantly to the photothermal AFM-IR technique and is a leader in expanding its applications. His contributions include the development of single-molecule and in-liquid AFM-IR imaging and spectroscopy, as well as the inaugural study on protein structure and aggregation with nanoscale IR spectroscopy.

Blending Physics, Biology, and Chemistry

Simone Ruggeri earned his PhD in the laboratory of physics of living matter at École Polytechnique Fédérale de Lausanne (EPFL) in Lausanne, Switzerland in 2015. His research there focused on protein aggregation and its role in neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases. This is where he first began to use atomic force microscopy (AFM) and nanoscale infrared spectroscopy (nanoIR) to understand the chemical properties of protein aggregates at the nanoscale.

Ruggeri was then awarded two research grants by the Swiss National Science Foundation, which he used to investigate single molecule and bulk properties of amyloids and protein aggregates at the University of Cambridge in the UK. He continued this line of study through a third grant as an independent Junior Research Fellow at the Darwin College in Cambridge. His work had implications both for disease research and for functional materials research.

In 2020, Ruggeri moved into his current role as a professor at Wageningen University (WUR) in the Netherlands, where he leads a talented group of graduate and post-graduate students to push forward the limits and capabilities of nanoscale microscopy and spectroscopy to unravel the properties of functional materials, biomolecular processes, protein science and neurodegenerative diseases.

Driving the Evolution of nanoIR for Protein Analysis

The technique of photothermal AFM-IR (or just AFM-IR) was commercialized in 2010 with the Anasys (now Bruker) nanoIR instrument, and Ruggeri researched on one of the first systems in Europe. Throughout his career, Ruggeri has been a significant contributor to the evolution of AFM-IR technology and its applications. His contributions are many, and only some are detailed here.

ABOUT THE RESEARCHER

Dr. Simone Ruggeri is an Associate Professor, leading the Laboratory of Nanoscale Microscopy and Spectroscopy within the Organic and Physical Chemistry Chair Groups at Wageningen University in the Netherlands.

- PhD in Natural Sciences, Swiss Federal Institute of Technology of Lausanne (EPFL), Switzerland
- Master's Degree in Physics & Materials Science, University of Catania, Italy
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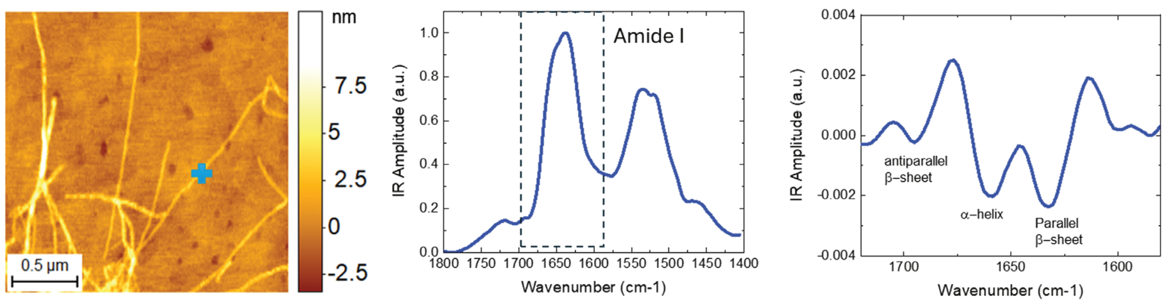
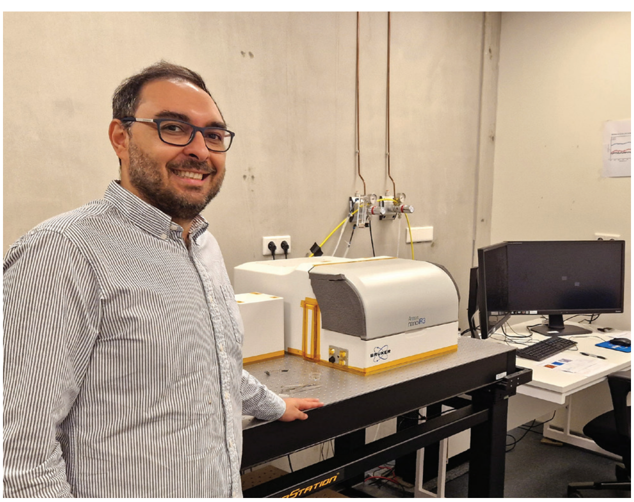


Single amyloid fibril secondary structure determination.

> "Using conventional AFM, it is very difficult to understand how proteins are changing structure during their functional versus pathological aggregation into fibrillar structures called amyloids. A universal cross beta-sheet signature of these aggregates seems fundamentally related to toxicity and the onset of neurodegenerative disorders. However, not only amyloid fibrils are formed, but there is a very, very complex and heterogeneous network of intermediate species whose properties differently relate to toxicity.
>
> So, it is very important to study all these heterogenous species one at a time to understand which physical and chemical properties could be related to the toxicity mechanism. However, these aggregates have nanoscale size and a highly heterogeneous nature. Using AFM-IR was a perfect tool to study them, because AFM-IR allowed us to probe one of these aggregates at the time and study chemical properties, things that were not possible with any other technology before."

Ruggeri was the first to apply photothermal AFM-IR in real-life problems in bioscience focusing on the study of protein aggregation.[1] In particular, he identified that proteins can first phase separate then aggregate via liquid-liquid phase separation, later showing how protein condensates evolve into aggregates.[2] Stemming from his initial research, there are now tens of groups in the world that use AFM-IR to study proteins, condensates, and aggregate formation in life function and malfunction.



Ruggeri with his nanoIR3 instrument.

Ruggeri then further developed the capabilities of his nanoIR2 instrument (Bruker) to achieve single-molecule sensitivity, enabling the characterization of protein secondary structures.[3]

> "I got my first nanoIR2 thanks to my collaboration with Anasys and Bruker. I pushed further the capabilities of this instrument to improve the sensitivity of the technology. One of the main milestones obtained with this instrument was reaching, for the first time worldwide, single-molecule sensitivity. With enough signal to not only identify a molecule, but also study its structural properties."

Leveraging the single-molecule sensitivity, Ruggeri and his co-authors studied the potential of AFM-IR in drug discovery, in the case of an FDA-approved specific drug able to inhibit aggregation and neurodegeneration in animal models.[4]

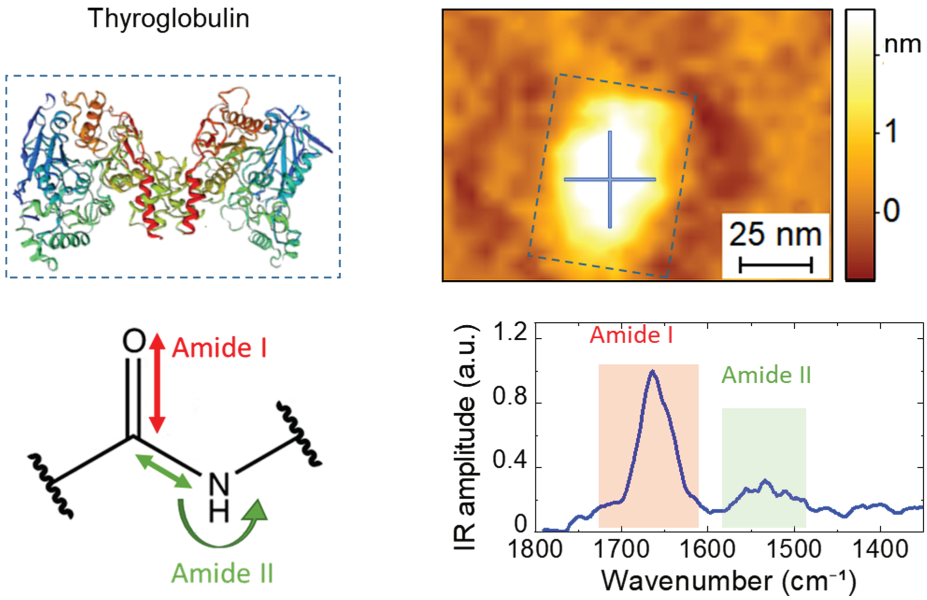
> "We used chemometrics to study the interaction of these amyloid aggregates with an approved drug that can stop amyloid formation in-vitro, but also in an animal model of Alzheimer's disease. AFM-IR allowed us to study drug-protein interaction down to the single molecular bond level."

In collaboration with NIST and Bruker, Ruggeri also developed liquid AFM-IR and used it to measure the structure of protein aggregates.[5] Sample preparation is another area where Ruggeri drove innovation; with several approaches, he demonstrated using a microfluidic spray to effectively prepare samples while preserving their molecular structure for analysis.[6,7]

> "That was key for us—the expertise of sample preparation is what allows us to put the technology on the forefront."

What is the role of proteins in neurodegenerative disorders?

Proteins need to fold in specific ways within our body to properly undertake their function. Whenever protein misfolding occurs, abnormal proteins can sometimes be corrected or cleared by natural processes. Otherwise, misfolded proteins can aggregate and have adverse effects. In Alzheimer's disease, for example, amyloid beta misfolding leads to insoluble aggregates called amyloid plaques, which build up outside of neurons and disrupt brain function.



Single-protein AFM-IR analysis.

Using AFM-IR for Diverse Applications

Ruggeri has investigated and will investigate a diverse range of materials and samples with AFM-IR, via newly awarded grants and key international collaboration, from perovskites and polymers to blood, cerebrospinal fluid (CSF) and human tissue.

Blood and CSF: the chemical information gained from protein aggregates in blood and CSF will be used in the frame of his newly awarded grant NanoNU-Marker to identify biomarkers of neurodegeneration in relationship to nutritional factors and lifestyle.

> "What happens nowadays is that we really have a biomarker of diseases such as Alzheimer's only several years after the disease has already struck. So, we really need more sensitive methods to detect earlier and with more information the onset of the disease. [P]rotein aggregates have been shown to be a very, very good biomarker to use; we will bring now their use as more sensitive biomarker via chemical detection at the nanoscale."

Perovskites: In perovskites used to produce solar cells, mapping the presence of additives with AFM-IR can drive efficiency improvements.

Polymers: AFM-IR enables the investigation of materials properties from the single-polymer chain to their properties in functional materials, such as current-transporting membranes.[8]

Micro- and Nanoplastics: Using AFM-IR, small plastic contaminants in food and drinking water can be characterized to predict and understand their health consequences.

Witnessing Research-Driven Impacts

It is particularly important to Ruggeri that his research contributions have made a meaningful impact, both to the research of others and to the broader community. He initially migrated after his MSc from materials science to biosciences, thus opening the possibility of having direct impact not only at a technological level but also on health-related societal problems.

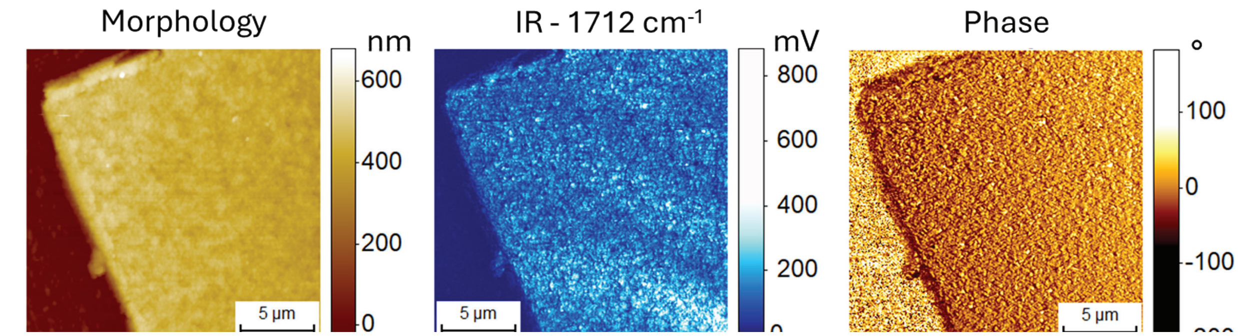
> "I wanted to apply my skill and my curiosity-driven approach [to] societal problems to improve quality of life."

Ruggeri has been told by researchers that his work became the inspiration for the focus of their own lab, which he finds energizing and promising, saying this collective push forward in the research is the only way to achieve challenging objectives.

What is photothermal AFM-IR?

Bruker's nanoIR instruments are based on the principle of photothermal AFM-IR, where photothermal expansion is locally induced by a laser and tracked using an AFM tip. This expansion directly relates to chemical structure, resulting in data output of spectra that can be analyzed in the same way as Fourier-transform infrared (FTIR) spectra. In contrast with FTIR, photothermal AFM-IR has nanoscale lateral resolution and the capability for high surface sensitivity.

Dr. Ruggeri receives his first AFM-IR in Wageningen.



Perovskite topography, IR map at 1712 cm^{-1}, and phase map.

Ruggeri routinely demonstrates his dedication to advancing AFM-IR applications and technology. He gives invited and plenary talks, participates on scientific panels (e.g., being part of the organisation of the european forum on IR nanospectroscopy), chairs nanoIR-related sessions every year at the SciX conference in the USA, and hosts hands-on workshops like the recent Summer School at the University of Orlando. He still even finds time to individually train his group members on the measurement process, which he believes is extremely important for staying connected with the technology.

> "It's very important to keep contact with the technology to understand how it's developing and how it could be developed further. What are the bottlenecks? What are the things that need to be improved? Then translating current technology and knowledge gaps into writing grants and projects to solve them."

Contributing to a Versatile AFM-IR Future

While Ruggeri remains a research powerhouse in protein science and AFM-IR technology advancement, he and his group are also making significant AFM-IR contributions in the field of materials science, with studies on perovskites, polymers, and micro-/nanoplastics.

> "My vision is to expand our nanoscale perception beyond imaging, to develop a nano-chemical angle of observation on challenging and puzzling scientific questions in a diverse range of fields of science."

Ruggeri presenting a talk on single-molecule AFM-IR.

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Ruggeri and his research group.

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