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Investigating the interaction between plasmonic nanoparticles and amyloid fibrils by surface enhanced Raman spectroscopy

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Summary. — In this contribution we provide nanoscale insights into the molecular interaction between insulin amyloid fibrils and gold nanoparticles (AuNPs) with different surface chemistry. By employing AuNPs as plasmonic nanoantennas and taking advantage of the high sensitivity and spatial resolution of surface enhanced Raman scattering, we identify the specific amino acids that are directly in contact with the nanoparticle surface. Our results emphasize the critical role of the surface chemistry in driving fibril disassembly, with distinct differences observed between the analyzed AuNPs.

1. – Introduction

The uncontrolled and irreversible aggregation of proteins into amyloid fibrils is nowadays widely recognized as a hallmark of several pathologies, including Alzheimer's and Parkinson's diseases and systemic amyloidosis [1], driving the development of innovative approaches not only to prevent amyloidogenesis but also to reduce mature amyloid structures. Recently, metallic nanoparticles (NPs) have gained attention as promising tools in this field. On one hand, they promote the disassembly of amyloid fibrils; on the other, their unique optical properties enable their use as local spectroscopic probes [2, 3].

The development of effective therapeutic strategies requires a detailed understanding of fibril-NP molecular interactions, down to the single amino acid level. To this aim, surface enhanced vibrational spectroscopies are highly sensitive techniques that allow probing biomolecular interactions at the nanoscale, relying on the optical properties of plasmonic nanomaterials. Noble metal NPs indeed, confine strong electromagnetic fields at the interface with the surrounding medium upon excitation of localized surface plasmons, within spatial regions below the diffraction limit [4]. As a result, the vibrational signal of molecules in direct contact with the metal experiences a significant enhancement, while that coming from more distant molecules remains undetected due to the rapid decay of the electromagnetic field within a few nanometers from the NP surface.

In this contribution, we exploit surface enhanced Raman scattering (SERS) to investigate the molecular interaction between gold nanoparticles (AuNPs) and insulin amyloid fibrils, identifying the spectral markers of the amino acids in direct contact with the metal surface. To analyze the role of the AuNP surface chemistry in these interactions, we conducted a comparative study using standard citrate-capped AuNPs as purchased; and AuNPs functionalized with the organic thiol 3-mercaptopropionic acid (3MPA), demonstrating how the characteristics of the molecular interface play a key role in driving the disassembly of mature fibrils.

2. – Materials and methods

Commercial AuNPs with a diameter of 60 nm and stabilized by a citrate capping, were purchased from Ted Pella, Inc. Monomeric native human insulin, 3MPA and all the other chemical compounds were purchased from Merck and used without further purifications. Insulin amyloid fibrils were obtained by dissolving insulin powder in Milli-Q water (pH 2, adjusted with 1 M HCl) to a final concentration of 430 μM. The solution was incubated at 65 °C for 12 hours under gentle stirring to induce fibrillation. The functionalization of AuNPs with 3MPA was performed using a well-established protocol based on ligand exchange [5]. Specifically, the citrate molecules capping the surface of the commercial AuNPs are replaced by 3MPA, whose thiol group (SH) covalently binds to the gold atoms on the NP surface through an S-Au bond.

Fibril-AuNPs samples were prepared by diluting the mature insulin fibrils in the AuNPs solutions with a ratio of 1:100 and incubating for at least 10 minutes at room temperature.

Raman and SERS spectroscopy measurements were performed using a Horiba HR-Evolution micro-spectrometer with a 15 mW He-Ne laser (632.8 nm) and a set of neutral density filters. The spectrometer was coupled with a confocal microscope, using a 100 $\times$ objective (NA 0.8, 1 μm laser spot) and a 600 groove/mm grating, with a spectral resolution better than 3 cm^{-1}. Spectral acquisition times varied between 3 and 20 s, depending on the sample. The laser power at the sample surface was 250 μW for SERS measurements and 15 mW for bulk fibrils. Samples for spectroscopy were obtained by depositing 20 μL of the sample dispersion on a glass slide and letting them dry at room temperature.

Atomic Force Microscopy (AFM) images were recorded using a Dimension Icon (Bruker AXS) instrument. Images were acquired in air, under ambient conditions, with a scan rate of 1 Hz, in tapping mode. A cantilever with a spring constant of 42 N/m and a tip with a nominal radius of curvature of 8 nm were employed. Samples for imaging were prepared by depositing 20 μL of the sample dispersion onto pre-cleaned silicon substrates and left them dry at room temperature. AFM images were analysed by Gwyddion software, version 2.65.

3. – Results and discussion

To investigate the molecular interaction between insulin amyloid fibrils and the different AuNPs at the nanoscale, we combined AFM and SERS measurements to assess both the morphological changes induced by AuNPs and the amino acids that drive the interaction with the AuNP surface. For this comparative study, we selected two types of AuNPs. Although both are coated with molecules featuring terminal carboxyl groups, citrate is weakly bound to the AuNP surface, making it more easily displaced by molecules with

a stronger affinity for gold [6]. In contrast, the S-Au bond formed by 3MPA is much stronger, hampering other molecules from easily interacting with the gold surface.

Representative AFM images of insulin amyloid fibrils interacting with citrate-AuNPs and 3MPA-AuNPs are shown in fig. 1, in comparison with the topography of mature fibrils. The images reveal that, upon interaction with both types of AuNPs, fibrils appear significantly shorter. In particular, fibrils interacting with citrate-AuNPs exhibit a higher degree of fragmentation compared to those interacting with 3MPA-AuNPs, highlighting the crucial role of the molecular interface in driving fibril disassembly. To obtain a quantitative comparison between the two samples, we analyzed the fibril length distribution by manually tracing fibrils in the acquired AFM images. The resulting length histograms, shown in fig. 1(d), indicate that the average fibril length is $\sim$ 320 nm for citrate-AuNPs, whereas for 3MPA-AuNPs it is $\sim$ 665 nm.

To analyze the interaction between fibrils and the two types of AuNPs at the molecular level, we performed SERS spectroscopy. First, we acquired the SERS spectrum of the AuNPs to characterize their intrinsic spectral features and identify vibrational bands arising from the molecular capping layers, which must be considered in the subsequent analysis of fibril interactions, as shown in fig. 2. The spectra of citrate-AuNPs are mainly characterized by the presence of a marked band at $\sim$ 1585 cm^{-1}, primarily associated with the asymmetric stretching vibration of the carboxyl groups [7]; and a band with two

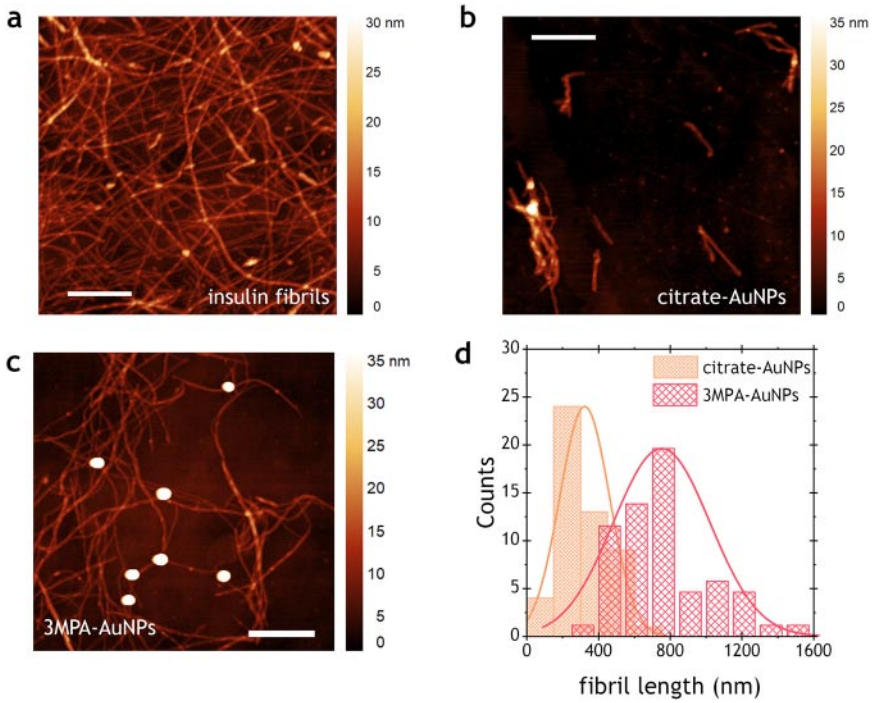

Fig. 1. – AFM characterization of fibril-AuNPs interaction. (a) AFM image of mature insulin amyloid fibrils. Fibrils following interaction with citrate-AuNPs (b) and 3MPA-AuNPs (c). The scalebars correspond to 500 nm. (d) Histogram of the length distribution of insulin amyloid fibrils interacting with citrate-AuNPs and 3MPA-AuNPs with the corresponding fit to a Gaussian distribution.

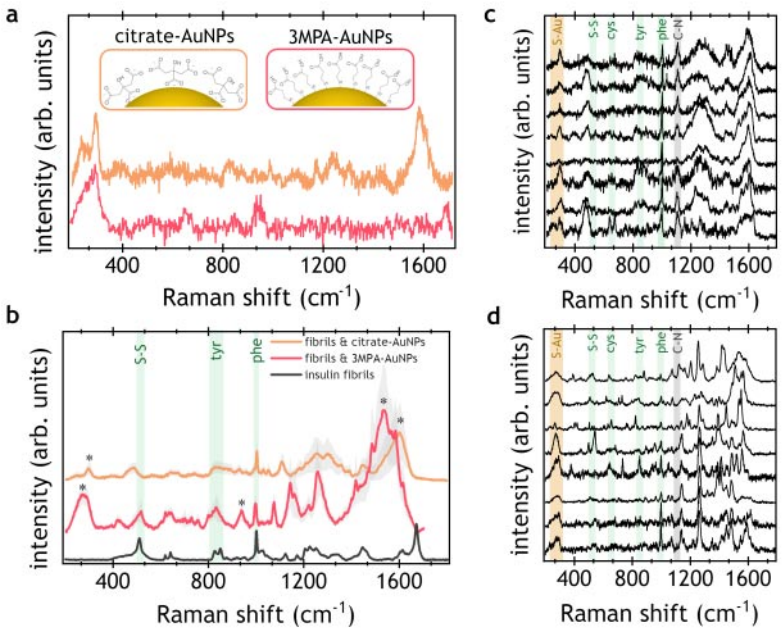

Fig. 2. – SERS analysis of fibril-AuNPs interaction. (a) SERS spectra of citrate-capped AuNPs (top) and 3MPA-AuNPs (bottom). The insets in the figure illustrate a schematic representation of the molecular interface of the two different AuNPs used in this study. (b) Average SERS spectra of insulin amyloid fibrils interacting with citrate-AuNPs (top) and 3MPA-AuNPs (middle), compared to the Raman spectrum of bulk insulin fibrils (bottom). All spectra are normalized to the peak at 1000 cm^{-1}, corresponding to the phenylalanine ring breathing mode. The gray shading around the spectra represents the standard deviation. Asterisks indicate spectral bands that can be confidently attributed to the background signal of the AuNPs. Selected SERS spectra acquired at different points on the sample for citrate-AuNPs (c) and 3MPA-AuNPs (d) interacting with insulin fibrils. Raman bands corresponding to amino acids and specific chemical groups are highlighted.

peaks at low wavenumbers ($\sim$ 238 and 298 cm^{-1}), which are attributed to the presence of adsorbed chlorides on the gold surface [7,8]. The SERS spectrum of 3MPA-AuNPs show the most prominent peak at $\sim$ 280 cm^{-1}, which corresponds to the S-Au bond marker. Other bands are observed at 655 cm^{-1} (ν(C-S)) and around 930 cm^{-1} (ν(C-CCO$^-$)), which are associated with the characteristic vibrations of the 3MPA molecule [9].

To gain a comprehensive understanding of the molecular interactions at play, we first computed the average of the SERS spectra acquired from various areas of the samples, for insulin fibrils interacting with the two types of AuNPs. The average spectra were then compared to the Raman spectrum of bulk fibrils, as shown in fig. 2(b). For both citrate-AuNPs and 3MPA-AuNPs, spectral contributions from the AuNPs molecular capping layers can be identified. This is an unavoidable effect due to the proximity of the capping molecules to the AuNPs surface. Nonetheless, spectral markers associated with the vibrational signature of amino acids are clearly identifiable, as the S-S stretching vibration around 515 cm^{-1}, which is attributed to cysteines (cys) arranged in disulfide bridges, as well as the ring breathing modes of tyrosine (tyr) and phenylalanine (phe) at

850 and 1000 cm^{-1}, respectively. It is not possible to straightforwardly identify other amino acids due to their analogous chemical structure, as in the case of amino acids characterized by the presence of aliphatic chains, whose vibrational modes occur around 1450 cm^{-1}. For a complete Raman spectral assignment of insulin fibrils, see ref. [10].

Regarding the S-S marker band, we notice its absence in the average spectrum of citrate-AuNPs, suggesting that disulfide bonds may be either absent or significantly reduced in these samples. This finding is consistent with previous observations in lysozyme amyloid fibrils [2]. Similarly, the band corresponding to the tyr Fermi doublet appears substantially flattened in citrate-AuNPs, suggesting that this amino acid may have limited interaction with the citrate-AuNPs. In contrast, phe marker peak remains clearly visible in both samples, partly due to its inherently higher Raman cross-section.

To obtain a more detailed picture of the interaction, we took advantage of the surface sensitivity of SERS and its nanoscale spatial resolution, by performing a point-by-point spectral analysis that provides additional insights into the heterogeneity of fibril-AuNP interactions. Since fibrils extend over several micrometers, while the SERS signal is localized only within the nanometric region where the fibril is in direct contact with the AuNP surface, their interaction is not homogeneous across the whole sample. As a result, averaged spectra may not fully capture site-specific molecular interactions. Representative spectra of fibrils interacting with citrate-AuNPs and 3MPA-AuNPs acquired in different regions of the samples are reported in fig. 2(c) and (d), respectively. The spectra exhibit noticeable variability in peak positions and intensity across different sampling points, highlighting the heterogeneity of fibril-AuNPs interaction at the nanoscale.

For the citrate-AuNP sample (fig. 2(c)), we observe the S-Au marker band in the low frequency spectral region which, combined with the near absence of S-S bands, suggests that cys residues are binding to gold. This chemical interaction underlies the observed fibril fragmentation, a phenomenon previously reported for other proteins [2]. Furthermore, cys related vibrational modes around 650 cm^{-1} appear in a significant number of spectra, reinforcing the hypothesis that this amino acid is in close contact with the gold surface. Conversely, in the 3MPA-AuNP sample, the analysis of single spectra reveals the persistent presence of S-S marker bands, indicating that intermolecular disulfide bonds remain intact in these samples, which correlates with the reduced fibril fragmentation. Therefore the presence of a covalently bound capping layer of 3MPA molecules prevents direct cysteine-gold interactions, thereby preserving the fibril structure.

4. – Conclusions

In this contibution, we investigated how the molecular surface chemistry of AuNPs impacts on the structural integrity of insulin amyloid fibrils at the nanoscale. By exploiting the high sensitivity and spatial resolution of surface enhanced Raman spectroscopy, we identified the specific amino acids mediating the interaction with the metal surface. Concurrently, atomic force microscopy revealed that the capping layer of AuNPs plays a decisive role in inducing fibril disassembly. Specifically, citrate-capped AuNPs promote extensive fibril fragmentation, a process driven by the weakly bound citrate molecules, which allow for direct gold-sulfur interactions with cysteine residues. In contrast, when AuNPs are functionalized with a covalently bound capping layer, such as the organosulfur compound 3MPA, fibril rupture is significantly reduced.

These findings underscore the critical role of AuNPs interface on amyloid fibril stability, with important implications for the design of nanomaterials for biomedical applications.

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