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Electronic Supplementary Information

Tip-enhanced Raman spectroscopy reveals structural rearrangements of tau protein aggregates at the growth phase

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Experimental Procedures

Preparation of samples

Gold substrate preparation: gold substrates for TERS measurements were prepared using a template-stripping method according to previously reported protocol by Banner et al.[1] First, freshly cleaved mica was coated with 200 nm of gold using a home-made evaporation system. A deposition of gold onto a mica (with a rate of 0.05 nm s^{-1}) was followed by a flame-annealing with a Bunsen burner powered by natural gas for c.a. 3 minutes. Afterwards, clean Si wafers were glued to flame-annealed gold surface deposited on mica with a thin layer of epoxy glue. Such prepared templates of gold substrate were left for at least 12 h under ambient conditions (to ensure the glue has hardened) and then stored under vacuum until use. Just before tau protein deposition, the Si wafer was stripped from the mica using a razor blade, revealing a contamination-free, ultra-clean and atomically flat gold surface on Si wafer.

Tau aggregation protocol

Tau protein was purchased from Abcam (ab256153). Tau protein was diluted to a final concentration of 10 µM in phosphate buffer containing 2.5 µM of heparin sodium obtained from TCI Europe N.V. (Tokyo Chemical Industry). The resulting solution of tau protein was incubated at 37 °C in the darkness for 336 h.

Tau protein deposition protocol

A 10 µL droplet of tau protein solution was deposited onto the freshly stripped gold substrate for 15 minutes at room temperature. Tau protein deposition was followed by rinsing with c.a. 2 mL water to remove the excess of the not adsorb tau protein. According to this procedure, samples for AFM imaging and TERS were prepared after 0, 24, 48, 72, 168, and 336 h of tau protein incubation/aggregation.

TERS probes preparation

AFM top visual probes VIT_P/IR (TipsNano) were coated with a) 5 nm of a Ti wetting layer, and b) 400 nm of Au. The coverage process of silicon probes with plasmonic nanostructures (metal layers) was performed using a home-made evaporation system under the pressure of 10^{-7} mbar with constant deposition rate of 0.05 nm s^{-1}.

Imaging with TERS probes

AFM topographies were collected prior TERS point-spectra acquisition/mapping using TERS probes. Images were acquired in tapping mode with a scan resolution of 256x256 pixels, and a scan rate of c.a. 0.4 Hz.

TERS experiments

After the selection of the area of interest based on the AFM topography, TER single-point spectra or TERS maps were acquired at manually selected locations. TERS measurements were performed using a SmartSPM™ 1000 Scanning Probe Microscope (HORIBA France SAS) microscope equipped with OmegaScope integrating the microscope with a Labram HR Raman spectrometer (HORIBA France SAS) with CCD camera (cooled to -79 °C). In all experiments a HeNe laser (633 nm) was applied. TERS spectra were acquired in the spectral range of 1800-650 cm^{-1} with a spectral resolution of 2 cm^{-1}. The time of spectra acquisition was 5-20 s depending on the signal-to-noise ratio (SNR). TERS map was acquired with 10x10 pixels, 10 nm per pixel, and 10 s of spectrum acquisition.

AFM imaging

AFM imaging was performed in air using a tapping mode with an image resolution of 1024 x 1024 or 512 x 512 pixels and scanning rates in a range 0.5-1 Hz. AFM images were acquired using the SmartSPM™ 1000 Scanning Probe Microscope (HORIBA France SAS) and RTESPA-150 probes (Bruker).

Data Processing

AFM images were flattened by a 1st order polynomial correction using Gwyddion software[2] (version 2.51).

Prior to the multivariate data analysis, the spectra were baseline corrected (3rd polynomial, 70 points), smoothed (polynomial, size 3, degree 4) and normalized (Standard Normal Variate (SNV) method) in the unique for biological molecules spectral range of 1800–800 cm^{-1}. When necessary, the removal procedure of cosmic rays was applied.

References

1 L. T. Banner, A. Richter, E. Pinkhassik, Surf. Interface Anal. 2009, 41, 49–55.

2 D. Nečas, P. Klapetek, Open Phys. 2012, 10, 181–188.