Electronic Supplementary Material (ESI) for Analyst.

Probing Amyloid Fibrils Secondary Structures by Infrared Nanospectroscopy: Experimental and Theoretical Considerations

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Supplementary Material:

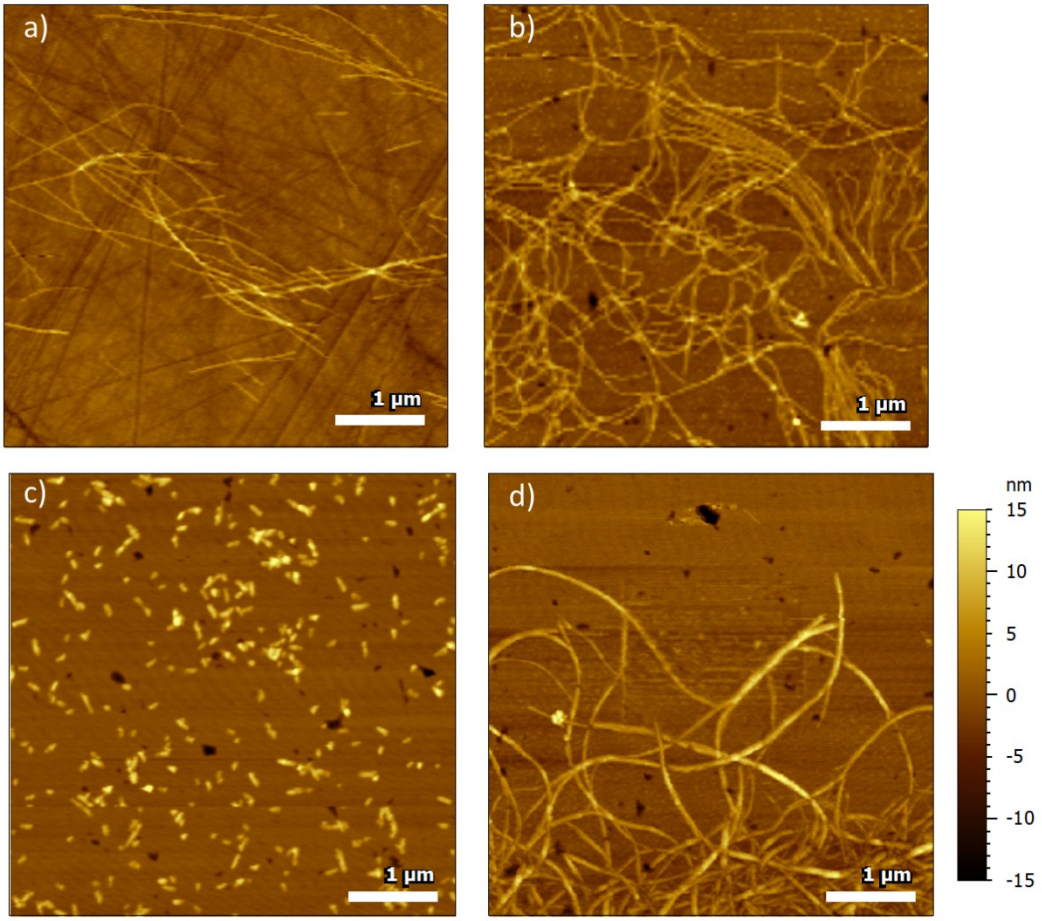

Figure SI 1. AFM topography of the different amyloid fibrils study in these articles obtained in contact mode with a Si-tip. a) α-syn; b) aβ; c) PrP and d) Hfq. Those images show isolated fibrils with a common feature: a diameter of around 10 nm.

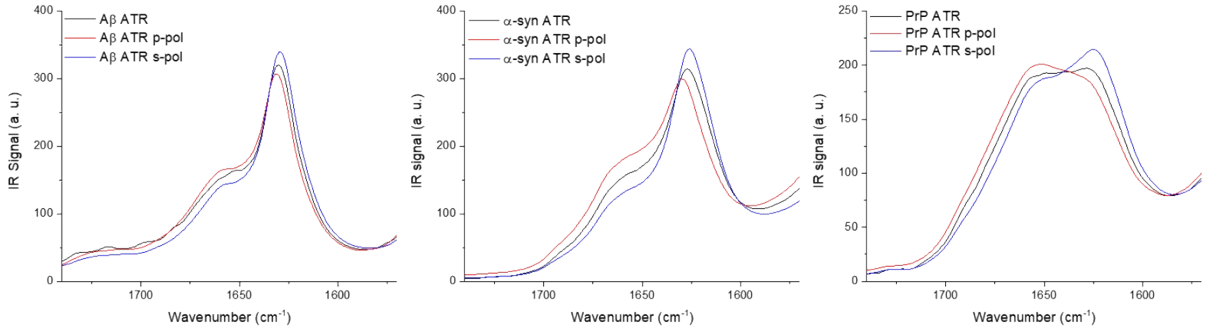

Figure SI 2. Polarized ATR-FTIR analysis of amyloid fibrils obtained without polarized (black curve) at s-(blue curve) and p-polarization (red curve). Normalized on amide I spectra of the 3 different proteins a) Aβ, b) α-syn and c) PrP.

Additional simulations with gold substrate show that this sort of substrate does not change the direction of the electric field (Figure SI 3) and has only an effect on the intensity distribution in p-pol. This confirms that the gold tip prevails on the electric field distribution

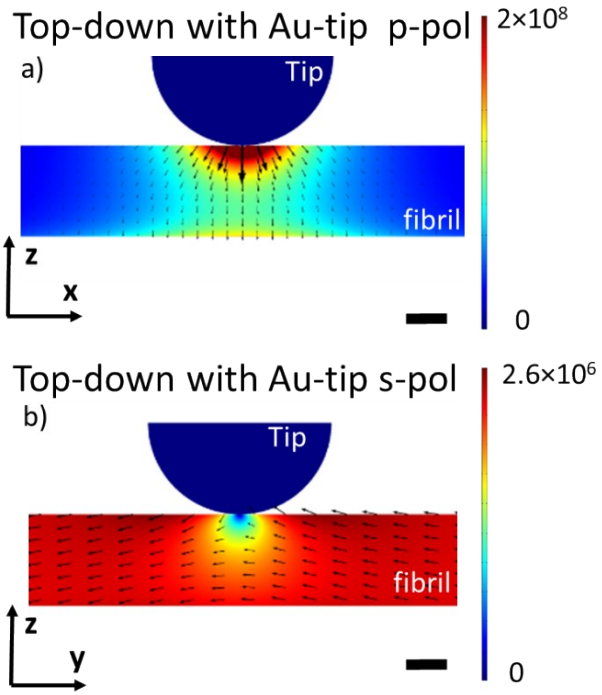

Figure SI 3. Finite elements simulation of the electric field induced in fibrils by Top-down illumination with Au-tip and gold substrate in a cross-section along the fibril axis: p-

polarization a) and s-polarization b). Electric field intensity (colored scale) and orientation (arrows) are represented only in the fibril. Scale bar 5 nm.

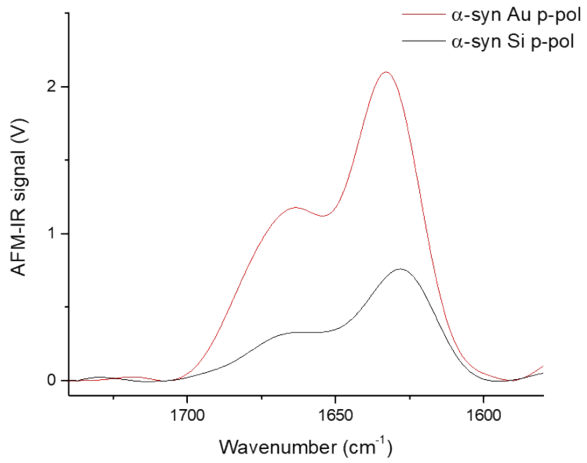

Figure SI 4. Effect of the enhancement of the Au-coating. Black curves correspond to Si-tip and red curves to Au-tip with a bottom-up illumination at p-pol and without scaling. The signal obtained with the Au-tip is around 2 times bigger than the signal with Si-tip.

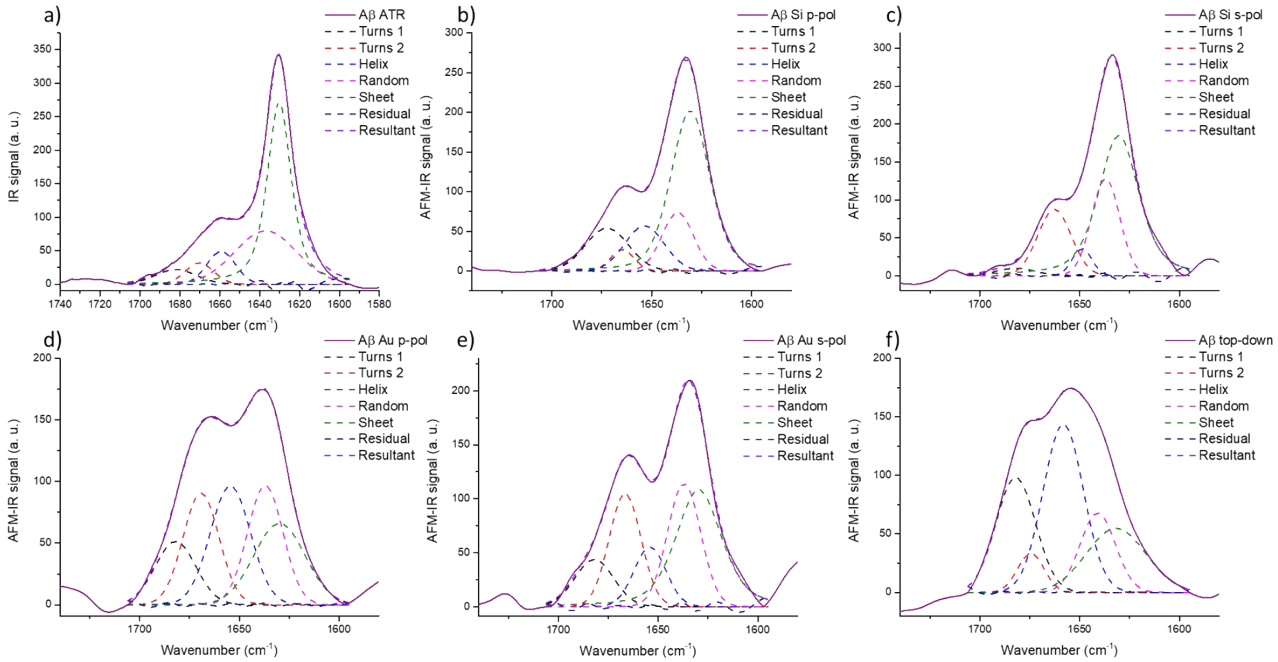

Figure SI 5. Secondary structure analysis of Aβ : a) ATR-FTIR, b) AFM-IR bottom-up configuration with Si-tip and p-pol, c) AFM-IR bottom-up configuration with Si-tip and s-pol,

d) AFM-IR with top-down illumination with Au-tip and p-pol, e) AFM-IR bottom-up configuration with Au-tip and p-pol and f) AFM-IR bottom-up configuration with Au-tip and s-pol. The colored dashed line corresponds to the curve fitting for the different components and solid line to the spectra.

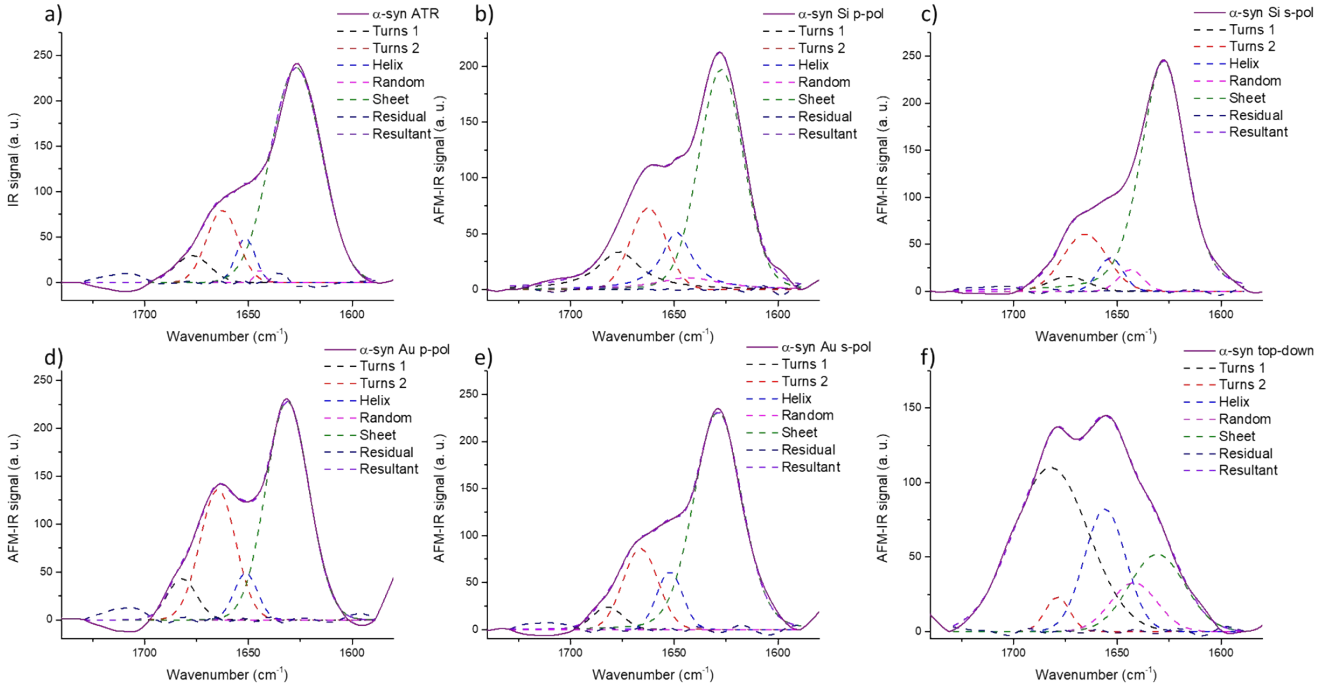

Figure SI 6. Secondary structure analysis of α-syn : a) ATR-FTIR, b) AFM-IR bottom-up configuration with Si-tip and p-pol, c) AFM-IR bottom-up configuration with Si-tip and s-pol, d) AFM-IR with top-down illumination with Au-tip and p-pol, e) AFM-IR bottom-up configuration with Au-tip and p-pol and f) AFM-IR bottom-up configuration with Au-tip and s-pol. The colored dashed line corresponds to the curve fitting for the different components and solid line to the spectra

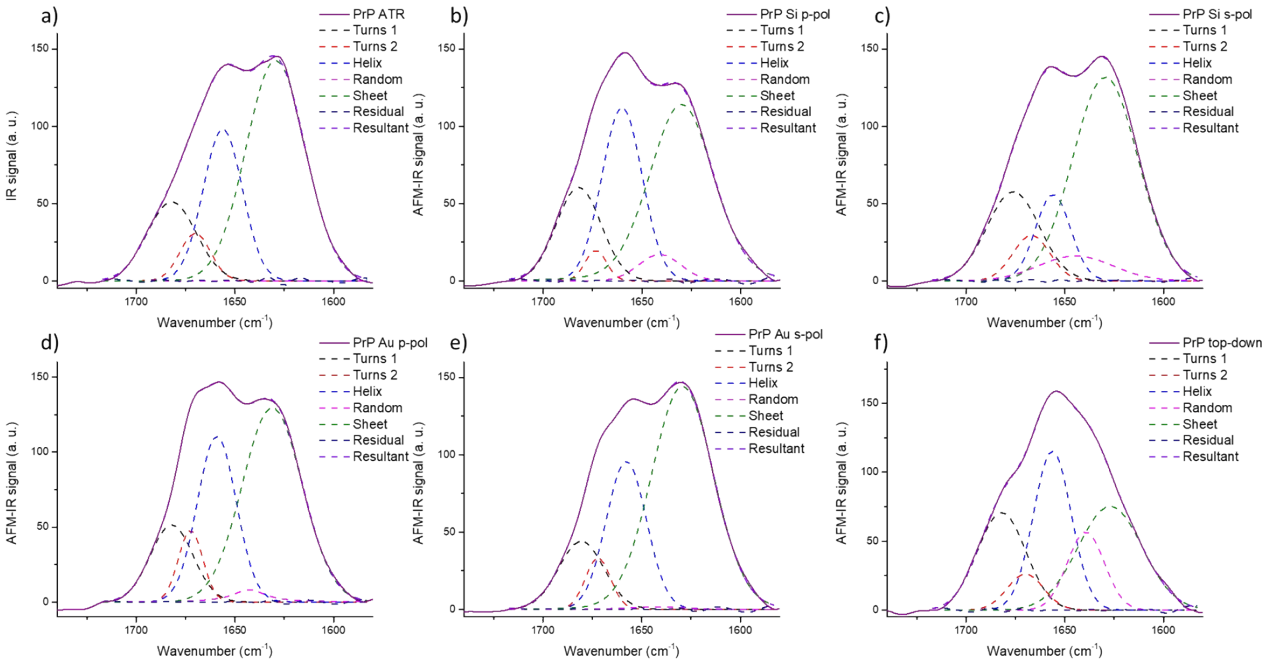

Figure SI 7. Secondary structure analysis of PrP : a) ATR-FTIR, b) AFM-IR bottom-up configuration with Si-tip and p-pol, c) AFM-IR bottom-up configuration with Si-tip and s-pol, d) AFM-IR with top-down illumination with Au-tip and p-pol, e) AFM-IR bottom-up configuration with Au-tip and p-pol and f) AFM-IR bottom-up configuration with Au-tip and s-pol. The colored dashed line corresponds to the curve fitting for the different components and solid line to the spectra

Aβ	ATR	Si p-pol	Si s-pol	Au p-pol	Au s-pol	Top-down
Beta	51	54	54	21	36	20
Random	30	14	22	21	23	15
Alpha	8	14	4	24	11	36
Turns	11	18	20	33	31	29

α-syn	ATR	Si p-pol	Si s-pol	Au p-pol	Au s-pol	Top-down
Beta	72	53	73	58	69	17
Random	1	6	3	0	0	9
Alpha	6	13	5	7	9	21

Turns	21	28	19	36	22	53

PrP	ATR	Si p-pol	Si s-pol	Au p-pol	Au s-pol	Top-down
Beta	54	48	54	49	57	31
Random	0	4	8	3	1	13
Alpha	24	28	12	27	24	28
Turns	22	20	26	21	18	28

Table SI 1. Results of the fitting for the secondary structure determination of amyloid fibrils with all the AFM-IR setup and ATR-FTIR spectra from Figure Si 2-4.

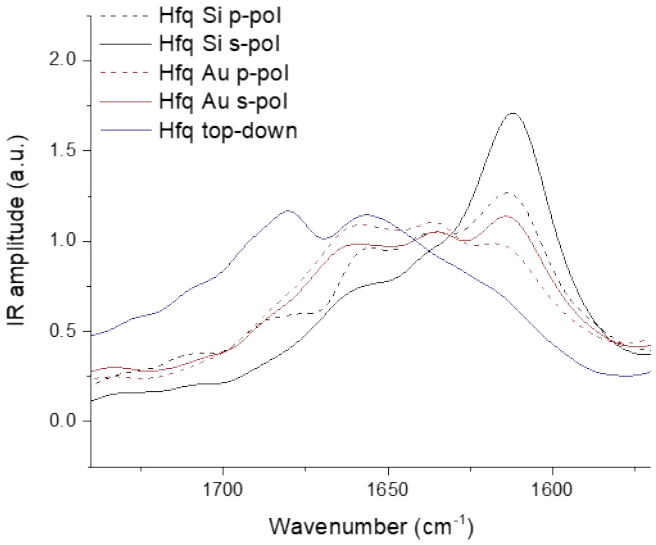

Figure SI 8. AFM-IR analysis of Hfq fibrils. Bottom-up illumination with Si-tip results are in black, with Au-tip in red and top-down illumination with Au-tip and p-pol in blue. The dashed line corresponds to p-pol and solid line to s-pol.