## Ultraviolet-visible-near-infrared optical properties of amyloid fibrils shed light on amyloidogenesis

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Supplementary Figure 1: Structure of Het-s prion domain amyloid fibres. (a) Wild Angle X-ray diffraction pattern of aligned Het-s prion domain amyloid fibres (Fig. 1). On the meridional 1D pattern (b), the typical signatures of the cross- $\beta$  structure of amyloid fibres manifest through reflections localized at 4.7 Å and 9.4 Å, respectively. The 1D equatorial pattern (c) is more complex than the one expected for a generic amyloid structure<sup>1,2</sup>. The additional reflection at 10-11 Å in the equatorial pattern combined with the reflection located at ca 4.7 Å and 9.4 Å in the meridional 1D pattern are indicative of the  $\beta$ -solenoid structure of the Het-s prion-domain<sup>2</sup>.



Supplementary Figure 2: Fluorescence microscopy on brain slices of APP<sub>Swe</sub>/PS1<sub>A246E</sub> mouse with A $\beta_{1.42}$  deposits, labelled with ThS ( $\lambda_{exc.} = 450$  nm,  $\lambda_{em.} = 488$ nm). Brain sections of AD mice were observed in hippocampus area, with emission in blue ( $\lambda_{exc.} = 390^{\pm 10}$  nm,  $\lambda_{em.} = 460^{\pm 50}$  nm) (a), green ( $\lambda_{exc.} = 475^{\pm 10}$  nm,  $\lambda_{em.} = 530^{\pm 50}$  nm) (b), and merging with transmission mode (c). Amyloid deposits are visible by ThS labelling (b).



Supplementary Figure 3: Fluorescence microscopy *ex vivo* on brain slices of 12 months old control mouse without A $\beta$  aggregates. Brain sections were observed in cortex area, in transmission mode (a), with emission in blue ( $\lambda_{exc.} = 390^{\pm 10}$  nm,  $\lambda_{em.} = 460^{\pm 50}$  nm) (b), green ( $\lambda_{exc.} = 475^{\pm 10}$  nm,  $\lambda_{em.} = 530^{\pm 50}$  nm) (c) and in NIR ( $\lambda_{exc.} = 620^{\pm 60}$  nm,  $\lambda_{em.} = 700^{\pm 75}$  nm) (d).



Supplementary Figure 4: Fluorescence microscopy *ex vivo* on brain slices of 15 months old control mouse. Brain sections were observed in cortex area, in transmission mode (a), with emission in blue ( $\lambda_{exc.}$ = 390<sup>± 10</sup> nm,  $\lambda_{em.}$ = 460<sup>± 50</sup> nm) (b), green ( $\lambda_{exc.}$ = 475<sup>± 10</sup> nm,  $\lambda_{em.}$ = 530<sup>± 50</sup> nm) (c) and in NIR ( $\lambda_{exc.}$ = 620<sup>± 60</sup> nm,  $\lambda_{em.}$ = 700<sup>± 75</sup> nm) (d).

## Supporting section: the blue luminescence characteristics

The blue luminescence is observed for all thirteen amyloid fibres investigated in this study (Table 1). This luminescent behaviour is illustrated with representative proteins (Fig. 2 and Supplementary Fig. 5) as well as for proteins involved in neurodegenerative diseases (Supplementary Fig. 6). The blue luminescence is detected independently of the aromatic side-chains present within the polypeptide<sup>3-9</sup>, as illustrated with the cases of i) lysozyme (Supplementary Fig. 5) which contains the three types of aromatic side-chains (Table 1), *ii*) insulin (Fig. 2) without tryptophan and iii) a peptide without aromatic side-chains (Supplementary Fig. 5). Although the luminescent behaviour is not identical for every amyloid fibre (Table 1), generic characteristics can be determined. The emission maxima are invariably located within the 430-450 nm spectral range when excited across the 340-380 nm wavelength domain. Moreover, they are dependent on the excitation wavelength for most fibres. Even if a weak blue luminescence is usually detected for the monomeric polypeptides, mainly for those containing tryptophan (Supplementary Fig. 5), a significant enhancement of the intensity is observed upon the microfiber formation (Table 1). Despite different behaviours of the emission spectra depending on the polypeptides, the excitation spectra are very similar with a band centred at ca. 350-370 nm (Fig. 2 and Supplementary Fig. 5). In some cases, the quality of the suspension allows to record an absorbance spectrum at high concentration. For lysozyme, a shoulder at 360 nm can be observed, which is barely seen in the case of the monomeric protein (Supplementary Fig. 5). A rough estimation of the molar extinction ( $\epsilon$ ) value for this transition gives a value of *ca*. 300 M<sup>-1</sup>.cm<sup>-1</sup>. In the case of monomeric lysozyme, a very rough estimation gives three times lower  $\varepsilon$  value in agreement with the change of the blue luminescence intensity (Supplementary Fig. 5 and Table 1). For the two other proteins which allowed such estimation, the  $\varepsilon$  values were on the same order of magnitude (Table 1). The observation of a blue luminescence in relation with the formation of amyloid fibres has been already reported in vitro<sup>3-7</sup>. All these data confirm a common characteristic of amyloid fibres, but the basic phenomenon at the origin of this luminescence remains poorly understood. Several tentative explanations have been proposed. Recently, the occurrence of quantum confinement due to the sub-10 nm size of the supramolecular assemblies<sup>8</sup> or charge delocalization within the H-bond network stabilizing the fibers<sup>3</sup> have been proposed. However, this blue luminescence has been reported for other types of polypeptide self-assemblies, such as crystals for instance<sup>9</sup>. At least for polypeptides containing aromatics, a contribution of their triplet state should be considered. Indeed, both

tryptophans and tyrosines show a phosphorescence, due to their triplet states, whose maxima of emission are within the same region (maxima at *ca.* 420-430 nm) as the blue luminescence<sup>13-15</sup>. This would explain the weak emission detected before the fibre formation. On the other side, the shift of the luminescence peak with the excitation wavelength is similar to a common observation with carbon quantum dots (CQD). This effect, so-called the giant red-edge effect (GRS), is related to environment effects on the charge delocalization at the origin of the luminescence<sup>10-12</sup>. A similar effect is observed on the intrinsic fluorescence emission of proteins when the excitation wavelength is higher than 300-310 nm (Supplementary Fig. 5g), *i.e.* on the red-edge of the excitation of tryptophans<sup>16</sup>. This effect can be used to investigate the local environment of tryptophans within proteins and their dynamics.



Supplementary Figure 5: *In vitro* absorption and luminescent properties of label-free amyloid fibres (a-c) Lysozyme and (d-f) NNLAIVTA peptide. (a&d) TEM images of amyloid fibres, (b&e) blue emission spectra of amyloid fibres (continuous lines:  $\lambda_{exc.}$ : 340 nm (pink), 360 nm (blue), 380 nm (cyan) and 440 nm (green)) and monomers (dashed lines: same colours); excitation spectra of amyloid fibres (dash-dot-dash lines:  $\lambda_{em.}$ : 430 nm (pink), 444 nm (blue) and 458 nm (cyan); absorbance spectra of amyloid fibres (black continuous line) and lysozyme monomers (black dashed line); (c&f) NIR signal of amyloid fibres (continuous line) and lysozyme monomers (dashed lines: same colours); excitation spectra (dashed line); (c&f) NIR signal of amyloid fibres (continuous lines of red scale colours:  $\lambda_{exc.}$ : from 600 nm to 670 nm and 680 in the case of NNLAIVTA peptide and monomers (dashed lines: same colours); excitation spectra of amyloid fibres (dash-dot-dash lines: the emission wavelengths correspond to the maximum emission wavelength). (g) Intrinsic fluorescence (tryptophan's) spectra of lysozyme amyloid fibres at various excitation wavelengths: (red) 260 nm, (orange) 270, (green) 280 nm, (cyan) 290 nm, (blue) 300 nm and (purple) 310 nm. (inset) changes of the maximum emission wavelength (Max Em.) as a function of the excitation wavelength (Exc.).



Supplementary Figure 6: Luminescent spectra of dispersions of  $\alpha$ -synuclein (a) and hTau (b) amyloid fibres in buffers. Excitation wavelength: 340 nm (pink), 360 nm (blue), 380 nm (cyan) and 440 nm (green), and from 600 to 670 nm (red colour scale). A multiplying factor of 30 was applied to the NIR emission signals.



**Supplementary Figure 7: Maximum emission wavelength** *vs* **excitation wavelength in the NIR.** Lysozyme (blue squares), insulin (green triangles down) and NNLVAITA peptide (red triangles up). The slope is equal to 1; indicating a constant Stokes shift.



**Supplementary Figure 8: (a)** NIR signal (normalized emission spectra) of Lysozyme amyloid fibres with excitation wavelengths from 630 nm (purple line) to 760 nm (brown line) by steps of 10 nm. (b) Stokes shift calculated from the excitation wavelength and the emission spectra (the colour scale is identical to that within panel (a)). (c) Influence of the emission slit on the NIR signal (excitation wavelength: 650 nm). The three spectra are recorded using different emission slit size of the spectrometer (4, 8 and 12 nm, for the purple, blue and green curves, respectively).



**Supplementary Figure 9:** Raman spectra of  $\alpha$ -lactalbumin as either monomer (black line) or amyloid fibres (red line). The band around 1000-1100 cm<sup>-1</sup> can be assigned to Phe side-chains, around 1200-1300 cm<sup>-1</sup> to amide III, around 1450 cm<sup>-1</sup> to CH<sub>2</sub> deformation, around 1550 cm<sup>-1</sup> to amide II and that around 1600-1700 cm<sup>-1</sup> to amide I<sup>17-18</sup>. The amide I band is centred on 1770 cm<sup>-1</sup>, as expected for an amyloid structure ( $\beta$ -strands).



**Supplementary Figure 10:** (a) blue luminescence ( $\lambda_{exc.} = 380^{\pm 5}$  nm and  $\lambda_{em.} = 450^{\pm 10}$  nm) and (b) NIR signal ( $\lambda_{exc.} = 640^{\pm 5}$  nm and  $\lambda_{em.} = 693^{\pm 10}$  nm) intensity of Lysozyme amyloid fibres *vs* Lysozyme concentration.



Supplementary Figure 11: Concentration and denaturation dependences of A $\beta_{1-42}$  amyloid fibres: (a) Luminescence intensity *vs* A $\beta_{1-42}$  fibril concentration (0-100 µM), in the blue ( $\lambda_{exc.}$  = 340 nm /  $\lambda_{em.}$  = 419 nm, blue O) and NIR ( $\lambda_{exc.}$  = 650 nm /  $\lambda_{em.}$  = 703, red O). (b) NIR Signal intensity of A $\beta_{1-42}$  fibrils ( $\lambda_{ex}$  = 640 nm,  $\lambda_{em.}$  = 695 nm) depending on time after adding HFIP, a denaturing agent for amyloid fibres.



Supplementary Figure 12: Kinetics of formation of amyloid fibres of insulin simultaneously monitored though (a) the blue emission (blue triangles:  $\lambda_{exc.}$ :  $360^{\pm 5}$  nm and emission intensity at 440 nm) and the NIR signal (red circles:  $\lambda_{exc.}$ :  $640^{\pm 5}$  nm and emission intensity at 695 nm), (b) Blue emission ( $\lambda_{exc.}$ :  $360^{\pm 5}$  nm) and (c) NIR signal ( $\lambda_{exc.}$ :  $640^{\pm 5}$  nm) spectra for increasing incubating times: 1,7 hr (pink), 2,4 hrs (blue), 2,8 hrs (green) and 3,5 hrs (red).



Supplementary Figure 13: Kinetics of formation of amyloid fibres of insulin simultaneously monitored through the ThT luminescence (green triangles:  $\lambda_{exc.}$ :  $440^{\pm 5}$  nm and emission intensity at 485 nm) and the NIR signal (red circles:  $\lambda_{exc.}$ :  $640^{\pm 5}$  nm and emission intensity at 695 nm).



**Supplementary Figure 14:** (a) NIR signal ( $\lambda_{exc.} = 640^{\pm 5}$  nm) of  $\alpha$ -lactalbumin amyloid fibres at a concentration of 30  $\mu$ M. (b) NIR signal ( $\lambda_{exc.} = 640^{\pm 5}$  nm) of a film made of  $\alpha$ -lactalbumin amyloid fibres. The film was prepared by drying a droplet of a solution at 3 mM of amyloid fibres. (c) Emission spectrum ( $\lambda_{exc.} = 655^{\pm 5}$  nm) of a Cyanine 5,5 solution at 3  $\mu$ M within a PBS buffer. At the exception of the excitation wavelength, all parameters used to record the spectra are identical.

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