

Structural and cellular Properties of Human Prion Protein

Oligomers

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Figure S1-S6

Supplementary Material

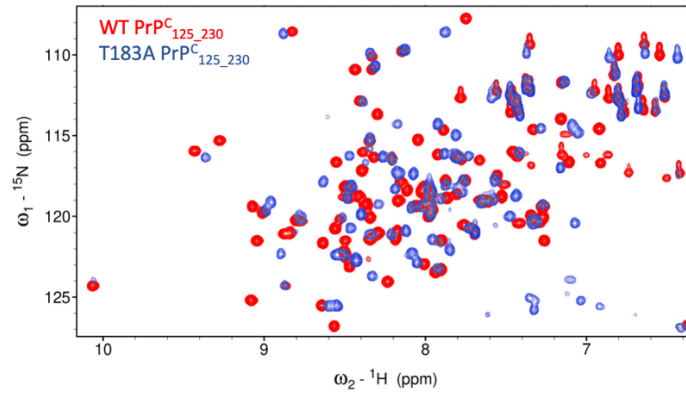


Figure S1. ${}^1\text{H}$ - ${}^{15}\text{N}$ -HSQC NMR spectra of WT (red) and T183A (blue) $\text{hPrP}^{\text{C}}_{125-230}$ measured in 100 mM phosphate buffer pH 7.0 at 16 °C on an 800MHz spectrometer. A single point mutation was shown to dramatically affect the spectral properties of this PrP domain, indicating changes in the folding stability and dynamics of this $\text{hPrP}^{\text{C}}_{125-230}$, as further confirmed by CPMG relaxation dispersion data ¹.

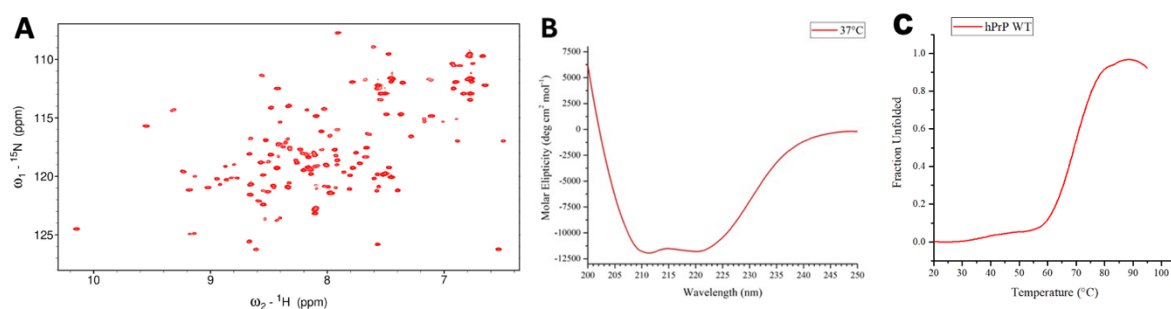


Figure S2. A) ^1H - ^{15}N -HSQC NMR spectrum of WT hPrP^C₁₂₅₋₂₃₀ measured in 100 mM phosphate buffer pH 7.0 at 37 °C on a 700MHz spectrometer, with a protein concentration of 100 μM . **B)** CD spectra of WT hPrP^C₁₂₅₋₂₃₀ measured at 37°C. **C)** Thermal denaturation curve of WT hPrP^C₁₂₅₋₂₃₀ measured in 100 mM phosphate buffer pH 7.0 reported in protein fraction unfolded against temperature.

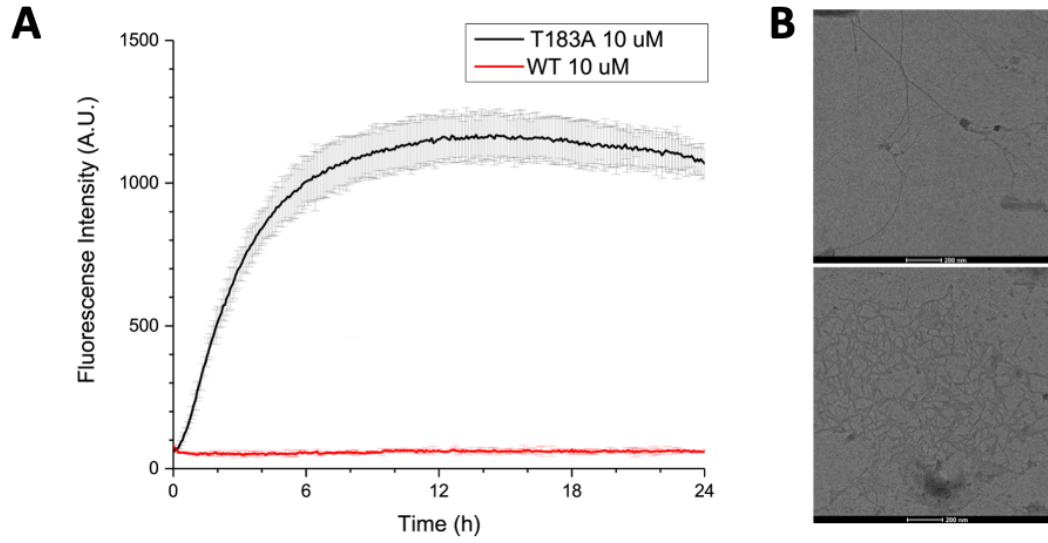


Figure S3. A) ThT fluorescence as a function of the time obtained by incubating WT (black) and T183A hPrP^C₁₂₅₋₂₃₀ at 37 °C, at a protein concentration of 10 μ M in PBS, using a double orbital shaking at 200 rpm following the conditions of our previous study ¹. **B)** TEM images of T183A hPrP^C₁₂₅₋₂₃₀ fibrils obtained after 48 hours of incubation at 37 °C, 200 rpm

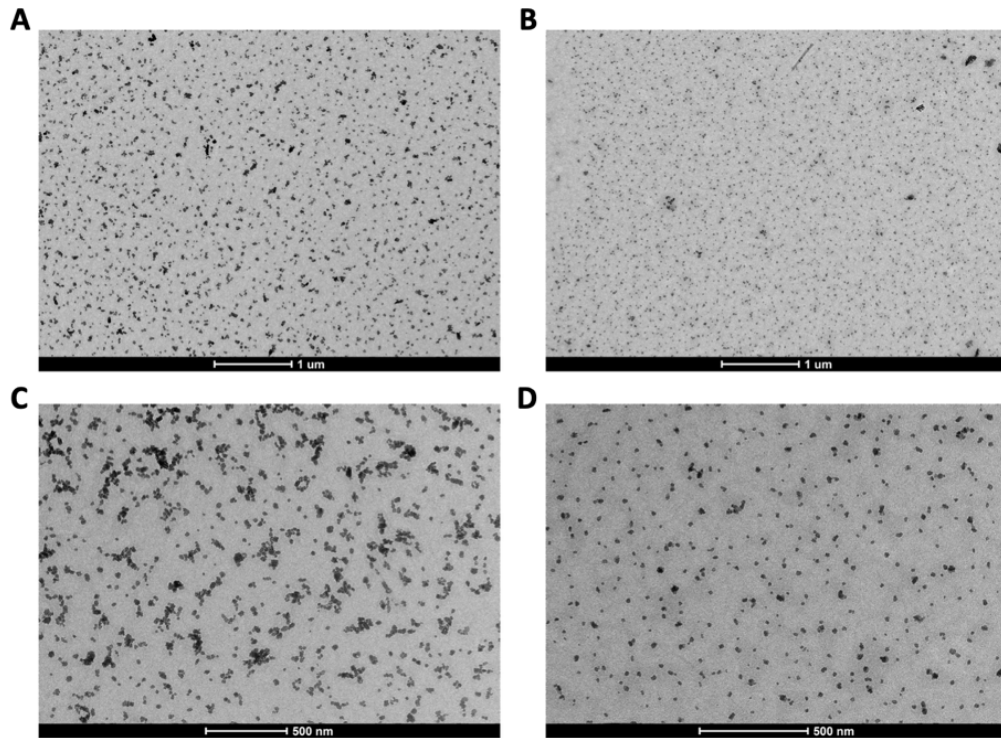


Figure S4. TEM images of hPrP₁₂₅₋₂₃₀ oligomer aggregates as freshly purified after 72 hours of incubation (panels A and C) and under further incubation in phosphate buffer at 37 °C for 1 week in quiescent conditions (panels B and D). The samples were stained with phosphotungstic acid. Scale bars are 1 μm (panels A and B) and 500 nm (panels C and D). The data show that the morphology and dispersion of the oligomers was not affected by the incubation post-purification.

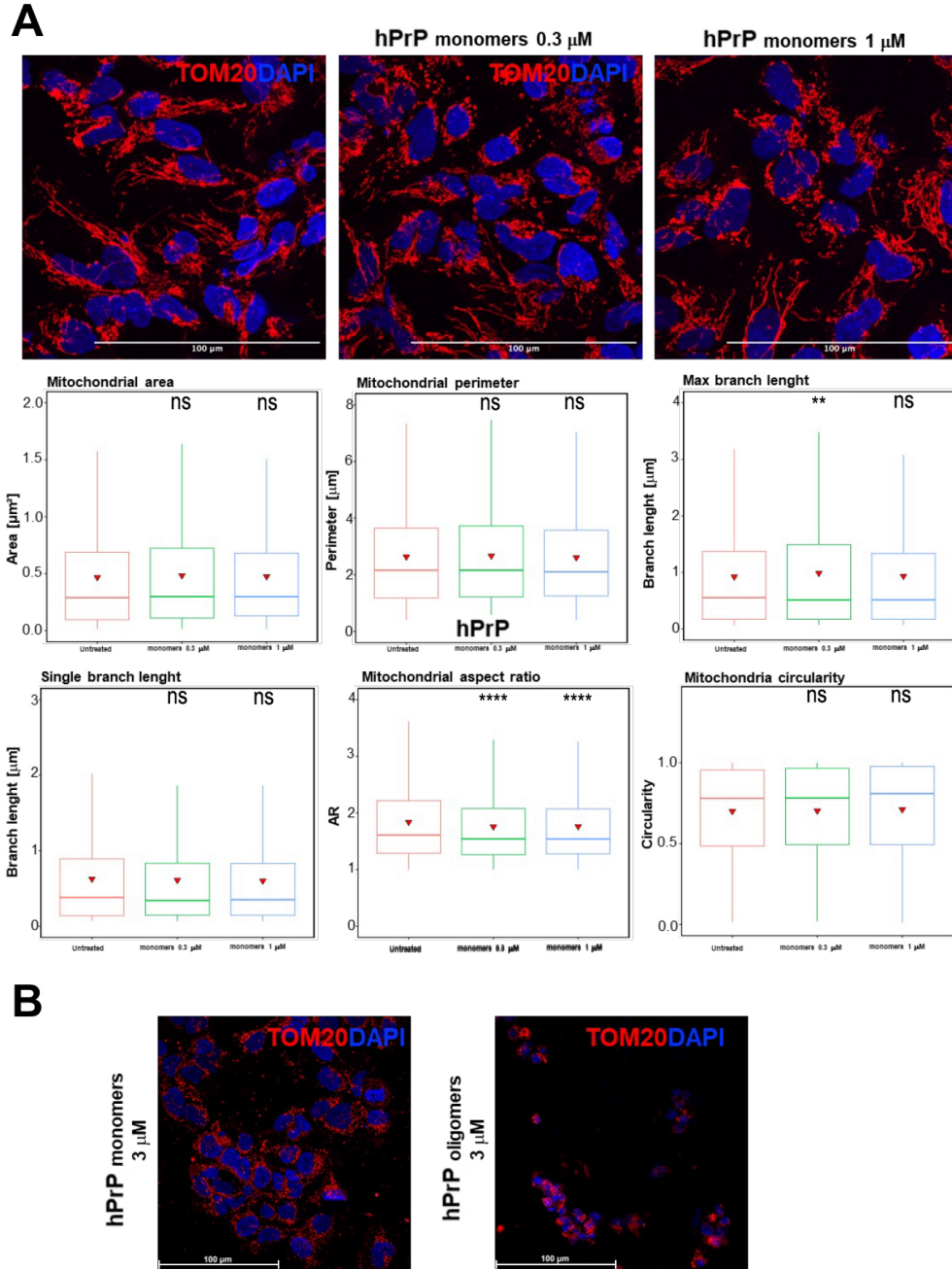


Figure S5. The treatment of NPCs with hPrP^C₁₂₅₋₂₃₀ monomers did not impact mitochondrial structure. (A) TOM20 immunofluorescence (red) on NPCs treated with increasing concentrations of hPrP^C₁₂₅₋₂₃₀ monomers and quantitative graphs relative to the dimensional analysis. DAPI (blue) was used to counterstain the nuclei. Maximum projection of z-slices is shown. Scale bar= 100 μ m. Box plots represent the interquartile range (IQR, 25th to 75th percentile), with the horizontal line indicating the median. The whiskers extend to the minimum and maximum values, and red triangles denote the mean for each group. Statistical comparisons were conducted using the unpaired t-test (ns = not significant, * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001). (B) Immunofluorescence images the effects of high doses of hPrP^C₁₂₅₋₂₃₀ monomers and oligomers (3 μ M) on NPC survival and mitochondrial structure. Scale bar= 100 μ m.

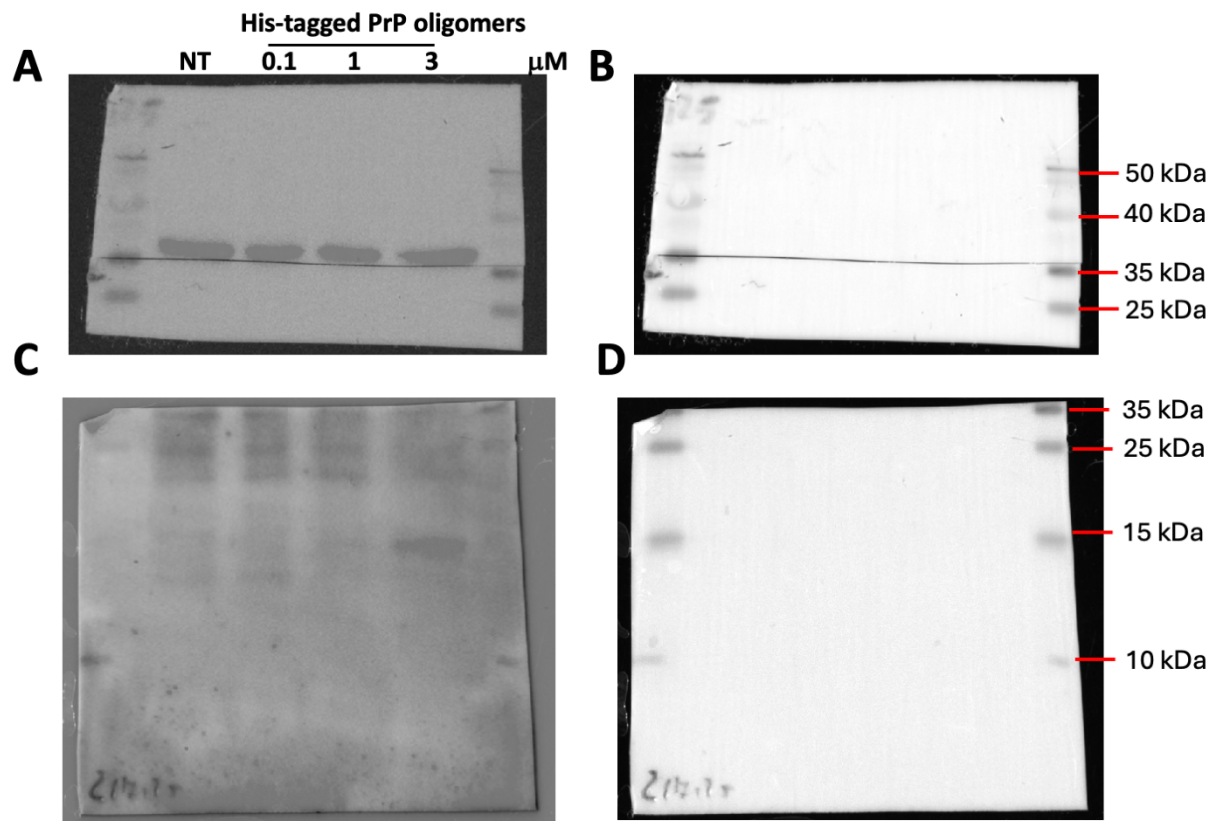


Figure S6. Raw Images of the western blot shown in Figure 5C. Western blot analysis of NPCs treated for 24h with different concentration of PrP oligomers (0.3, 1, 3 μM). Untreated (NT) NPCs were used as negative control. Gapdh was used as internal loading control. A, C) Merge of the chemiluminescence and colorimetric images. B, D) Colorimetric Images. A, B) The upper part of the membrane was used to detect Gapdh. C, D) The lower part was used to detect the His-tagged form of hPrP^C₁₂₅₋₂₃₀.

References

1. Sanz-Hernandez, M. et al. Mechanism of misfolding of the human prion protein revealed by a pathological mutation. *Proc Natl Acad Sci U S A* **118** (2021).