Revealing structural changes of prion protein during conversion from α-helical monomer to β-oligomers by means of ESR and nanochannel encapsulation

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Supporting Information

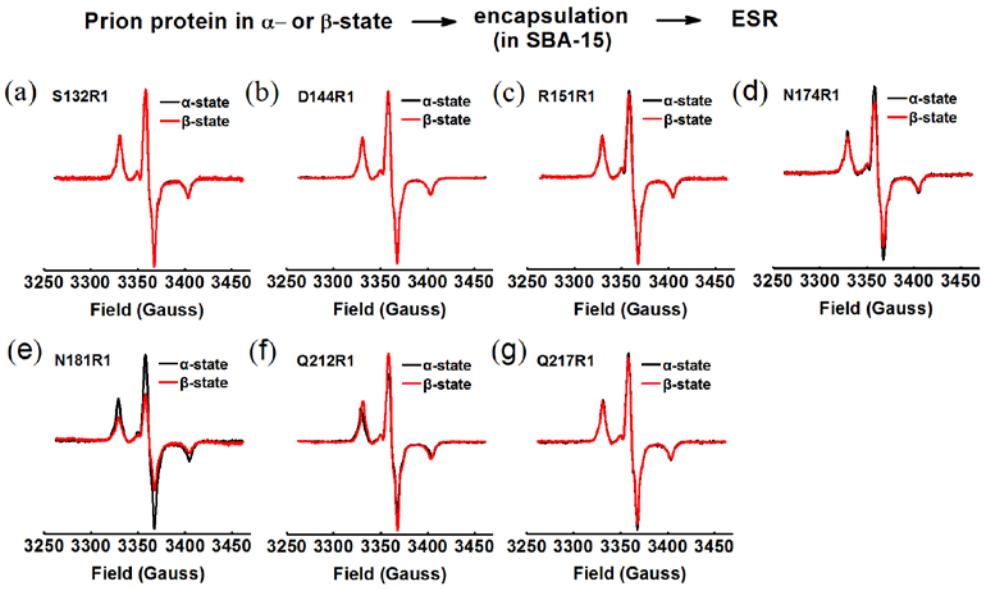

Figure S1. Cw-ESR spectra of single-labeled mutants in the α-state or β-state. The buffer used to prepare proteins in the α- or β- state was 0.5 mM NaOAc (pH 5) or 10 mM NaOAc (pH 7), respectively. S132R1, D144R1, R151R1, N174R1, N181R1, Q212R1, and Q217R1 were encapsulated in SBA-15 and their cw-ESR spectra in the α-state (black) or β-state (red) were recorded at 200K.

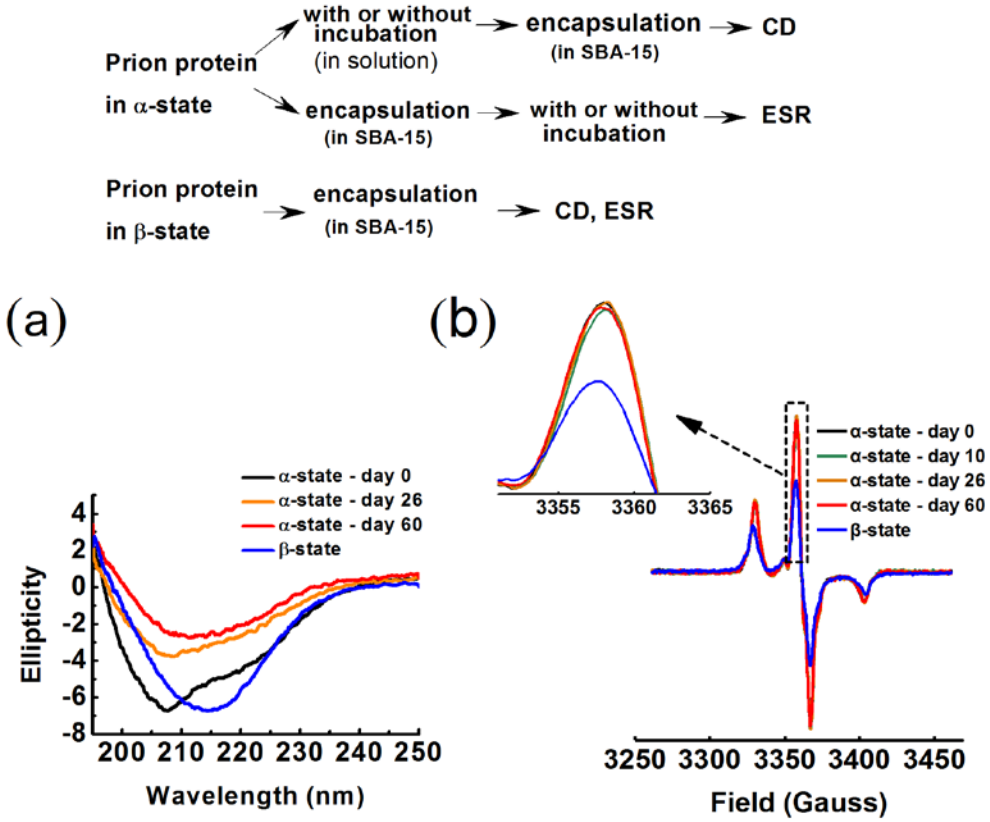

Figure S2. Conformational changes in N174R1 in buffer or in nanochannels. To prepare N174R1 in the α- or β-state, N174R1 was dissolved in, respectively, 0.5 mM NaOAc (pH 5) or 10 mM NaOAc (pH 7) at a final protein concentration of 10 mg/mL. (a) CD spectra of N174R1 in the α-state (black) or β-state (blue) recorded immediately after preparation and of the α-state N174R1 after incubation at 4 ºC for 26 or 60 days in buffer. An aliquot of the N174R1 in the α-state or β-state was encapsulated in SBA-15 nanochannels, and then the CD spectrum was recorded (marked as "day 0 for α-state). The rest of the α-state N174R1 solution was incubated at 4 ^{o}C. At the indicated day, an aliquot of protein solution was

encapsulated in SBA-15 nanochannels, and the protein structure was checked by CD spectroscopy. (b) Cw-ESR spectra of α-state N174R1 (fresh or incubated in nanochannels at 4 ^{o}C for 10, 26 and 60 days) and of β-state N174R1 in nanochannels. The cw-ESR spectra were measured at 200 K. These CD results show that N174R1 in buffer was converted from α-state to β-state within 60 days, whereas the corresponding cw-ESR spectrum was distinctly sharper than the β-state spectrum (blue), indicating that, after 60 days, N174R1 in nanochannels could not associate with other PrP molecules and was a β-PrP monomer.

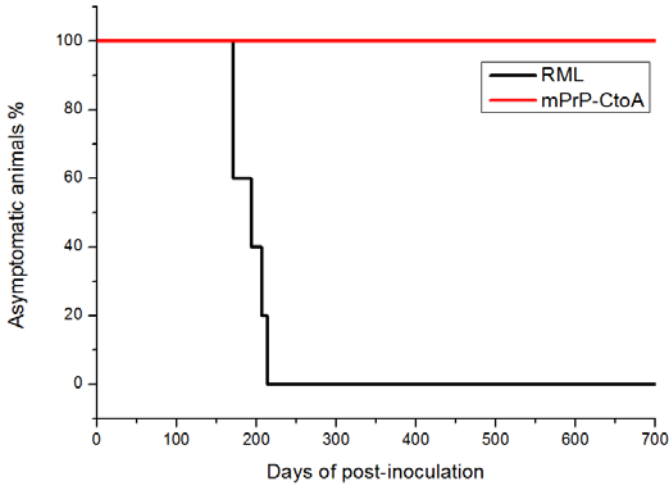

Figure S3. Survival graphs for C57BL/6 mice inoculated with RML-prion or β-oligomers. The mice were inoculated intracerebrally with 30 μL of brain homogenate from a RML-infected mouse (n=5) or 30 μL of β-oligomers formed from the disulfide bridge-lacking PrP protein mPrP-CtoA (0.1 mg/mL) in 10 mM NaOAc (pH 7) (n=4).

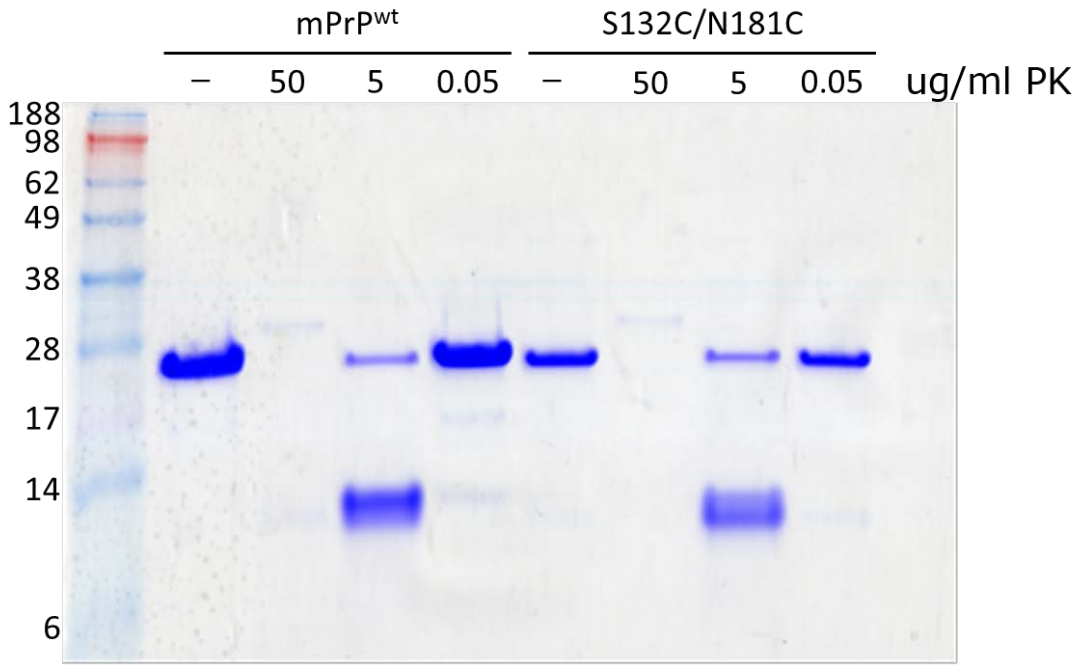

Figure S4. Protease K digestion assay. mPrP^{wt} or S132C/N181C was dissolved in 10 mM NaOAc buffer (pH 7) at a protein concentration of 0.8 mg/mL. mPrP^{wt} is a monomer, while S132C/N181C forms β-oligomers in this buffer. The protein samples were incubated at 37 ^{o}C for 1 h with the indicated concentration of recombinant proteinase K (Roche, Basel, Switzerland), then the reaction was terminated by addition of 5 mM PMSF and the samples analyzed by 12% SDS-PAGE.