SUPPLEMENTAL INFORMATION

EXTENDED EXPERIMENTAL PROCEDURES

NMR spectroscopy and structure calculations

Samples for NMR spectroscopy were prepared at a protein concentration of ~1 mM in 10 mM acetate buffer containing 0.005% sodium azide, protease inhibitors and 5% (v/v) $^{2}H_2O$, pH 4.6. NMR spectra were recorded at 26 ºC on Varian INOVA spectrometers operating at ^{1}H resonance frequencies of 500, 600, and 900 MHz or a Bruker Avance spectrometer operating at a ^{1}H resonance frequency of 800 MHz. Conventional (Cavanagh et al., 1996) and reduced dimensionality (Szyperski et al., 2002) spectra were recorded for resonance assignments. Backbone and side chain resonance assignments were obtained from HNCA, (H)C-TOCSY-(CO)NH, H(C-TOCSY-CO)NH, HC(C)H-TOCSY, RD HBCB(CGCD)HD, and RD ^{1}H-TOCSY-HCH-COSY. Additional resonance assignments and conformational restraints were obtained from ^{15}N resolved [^{1}H, ^{1}H]-NOESY (70 ms mixing time), ^{13}C resolved [^{1}H, ^{1}H]-NOESY (80 ms mixing time) optimized for aliphatic resonances, ^{13}C resolved [^{1}H, ^{1}H]-NOESY (70 ms mixing time) optimized for aromatic resonances, and a 2D [^{1}H, ^{1}H]-NOESY (70 ms mixing time). The programs PROSA (Guntert et al., 1992) and XEASY (Bartels et al., 1995) were used for data processing and data analysis, respectively.

NOE assignments were obtained through combined manual assignments and automated assignments using CYANA 2.1 (Herrmann et al., 2002). Dihedral angle constraints were derived from chemical shifts of residues in regular secondary structure elements using TALOS (Cornilescu et al., 1999). The final round of CYANA calculations was started with 100 randomized conformers and used 20000 steps and 1403 conformationally restricting distance constraints derived from 3D heteronuclear resolved and 2D homonuclear [^{1}H, ^{1}H]-NOESY spectra. The experimental data is well represented by the best 20 structures (out of 100 calculated) as indicated by the low CYANA target function, low RMS

deviations, and absence of constraint violations (Table S1). This ensemble was subjected to a water refinement (Nederveen et al., 2005) using Cartesian space simulated annealing with the program CNS, employing the distance and dihedral angle constraints that were used in the CYANA calculations. Results of the CNS refinement are summarized in Table 1. The water refined ensemble from CNS has been selected to represent the solution structure of V209M HuPrP. All structural figures were prepared with the program MOLMOL (Koradi et al., 1996).

Cavity volumes and surfaces were obtained with the program MOLMOL, calculating the contact surfaces with a probe radius of 1.4 Å. Reported are the values for wild type HuPrP (11) (PDB ID 1QM0), and the lowest energy structure of the V209M HuPrP ensemble. Similar results are obtained for calculations using other conformers in the ensemble.

Release of PrP from cell surface by PI-PLC treatment

Wild type human PrP-129M (HuPrP) ORF and the mutant PrP^{V209M} ORF were subcloned into the pCEP4 plasmid (Invitrogen, Carlsbad, CA) and transfected into human neuroblastoma M17 cell line with lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After selection with 400 μg/ml hygromycin B, the surviving cells were stained with anti-PrP antibody 3F4 (Covance, Princeton, NJ) and goat anti-mouse IgG (H+L) conjugated with Alexa fluor 488 (Invitrogen, Carlsbad, CA), followed by fluorescent-activated cell sorting to enrich for PrP-expressing cells. The cells were cultured overnight in 5% FBS in Opti-MEM with 1% penicillin and streptomycin and washed with Opti-MEM without serum. The cells were incubated with or without 0.1 unit/ml phosphatidylinositol-specific phospholipase-C (PI-PLC) (Invitrogen, Carlsbad, CA) in fresh medium for 45 min at 37 ºC. The medium was collected and centrifuged at 290 x g at 4 ºC for 10 min. Proteins in the supernatant were precipitated with 5 volumes of cold methanol at -20 ºC for at least 2 h, resuspended in 150 μl Laemmli sample buffer, fractionated by 15% Tris-HCl SDS-PAGE (Bio-Rad, Hercules, CA) and blotted onto PVDF membrane. The membrane was blocked in by 10% nonfat dry milk in TBS

buffer with 0.1% Tween 20, then incubated with primary antibody 3F4 and secondary antibody NA931V (GE Healthcare Bio-Sciences, Piscataway, NJ) diluted at 1:5000 in 0.5% Normal Goat Serum in TBS buffer with 0.1% Tween 20. The membrane was developed using BM Chemiluminescence Blotting substrate POD (Roche Applied Sciences, Indianapolis, IN).

Immunostaining of cell surface PrP before and after PI-PLC treatment

M17 cells were transfected with wild type human PrP or HuPrP^{V209M} and selected as described above. For PI-PLC digestion treatment, the cells were seeded at 70% confluency in LabTek Chamber Slides (Thermo Fisher Scientific, Rochester, NJ), left overnight, washed with PBS three times, and then incubated with PI-PLC (0.25 unit/ml) in PBS at room temperature for 3 h before immunostaining the cell surface PrP with 3F4 as described above.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Differential scanning calorimetry curves for HuPrP90-231 and the V209M variant, Related to Figure 1

Experiments were performed on a Microcal VP-DSC system (scanning rate of 60 degree/h) at a protein concentration of 43 μM in 50 mM sodium acetate buffer, pH 4. Under these conditions, the calorimetric curves were reversible, as indicated by nearly identical curves observed in sequential scans. The DSC curves could be fitted to a two-state transition model. The midpoint denaturation temperature, Van't Hoff enthalpy and calorimetric enthalpy for the wild-type protein are 56.4 ^{O}C, 197.5 kJ/mol and 197.9 kJ/mol, respectively. For the V209M variant, the respective parameters are 62.5 ^{O}C, 266.1 kJ/mol and 260.2 kJ/mol. At neutral pH thermal denaturation of both proteins was irreversible, precluding any thermodynamic analysis.

Figure S2. Line representation of the final ensemble of 20 superimposed, water refined CNS structures of V209M human PrP, Related to Figure 2

Only residues 125-231 are displayed as the N-terminal region is unstructured and flexible.

Figure S3. HuPrP^{V209M} is expressed at cell surface as a GPI-anchored protein, Related to Figure 3

(A) Western blot analysis of PrP released into the medium by phosphatidylinositol-specific phospholipase C (PI-PLC). Neuroblastoma M17 cells stably transfected with HuPrP or HuPrP^{V209M} were treated (+) or not treated (-) with PI-PLC, and the culture medium was collected and subjected to Western blot analysis with mAb 3F4.

(B-D) Immunofluorescence of cell surface PrP before and after PI-PLC treatment. HuPrP transfected M17 cells (B and C) and HuPrP^{V209M} transfected M17 cells (D and E) were either treated (C and E) or not treated (B and D) with PI-PLC and subjected to confocal microscopy examination after staining with mAb 3F4 in conjunction with goat anti-mouse IgG (H+L) conjugated with highly cross-absorbed Alexa fluor 488. Scale bars: 10 μm.

Figure S4. Histopathology and PrP^{Sc} deposits in sCJD-infected Tg($HuPrP^{V209M}$) and Tg(HuPrP) mice, Related to Table 1

(A-B**)** PrP immunostaining with mAb 3F4 in the cerebral cortex. Immunostaining is conspicuous in Tg(HuPrP) mice (A), but it is almost undetectable in the same brain region of Tg($HuPrP^{V209M}$) mice (B). Very limited PrP immunostaining is occasionally seen in some brain stem regions of Tg($HuPrP^{V209M}$) mice (inset).

(C**)** Lesion profiles in Tg($HuPrP^{V209M}$) mice after serial passages of sCJDMM1. The inoculums in the second to fourth passages were brain homogenates from Tg($HuPrP^{V209M}$) mice infected in a preceding passage. Cx, cerebral cortex; Sept n, septal nuclei; HI, hippocampus; BG, basal ganglia; TH, thalamus; BS, brain stem; CE, cerebellum.

(D**)** Lesion p rofiles after serial passages of sCJDMM1 in Tg(HuPrP) mice. Red triangles represent primary passage in Tg(HuPrP) mice. The inoculums in the second, third and fourth passages were sCJDMM1 prions passaged once, twice or three times, respectively, in Tg($HuPrP^{V209M}$) mice .

(E**)** Western blot of PK-treated PrP^{Sc} (using a standard pre-cast gel) as probed with mAb 3F4. Lane 1, primary passage in Tg($HuPrP^{V209M}$) mice; lanes 2-3, secondary passage in Tg($HuPrP^{V209M}$) mice after primary passage in Tg($HuPrP^{V209M}$) mice; lanes 4-5, secondary passage in Tg(HuPrP) mice after primary passage in Tg($HuPrP^{V209M}$) mice; lane 6, primary passage in Tg(HuPrP) mice; lanes 7-8, tertiary passage in Tg($HuPrP^{V209M}$) mice after two passages in Tg($HuPrP^{V209M}$) mice; lanes 9-10, tertiary passage in Tg(HuPrP) mice after two passages in Tg($HuPrP^{V209M}$) mice; lanes 11-12, quaternary passage in Tg($HuPrP^{V209M}$) mice after three passages in Tg($HuPrP^{V209M}$) mice; lanes 13-14, quaternary passage in Tg(HuPrP) mice after three passages in Tg($HuPrP^{V209M}$) mice; lane 15, sCJDMM1 control; lane 16, sCJDMM2 control. To better visualize the differences in electrophoretic mobility of PrP^{Sc} from Tg(HuPrP) and Tg($HuPrP^{V209M}$) mice, samples from the third passage in these mice [in each case after two passages in Tg($HuPrP^{V209M}$) mice] were re-run using a hand-cast long gel. A section of Western blot from this gel corresponding to non-glycosylated PrP^{Sc} is shown at the right side, with lanes 17 and 18 representing PrP^{Sc} from the third-passage in Tg($HuPrP^{V209M}$) and Tg(HuPrP) mice, respectively.

Table S1. Statistics of the NMR structural ensemble of V209M human PrP, Related to Figure 2

Distance constraints	1403
Intraresidue ($\|i - j\| = 0$)	441
Sequential ($\|i - j\| = 1$)	376
Medium range ($2 \leq \|i - j\| \leq 4$)	264
Long range ($\|i - j\| \geq 5$)	322
Dihedral angle constraints	124
ϕ	62
ψ	62
CYANA target function [$Å^2$]	0.12 ± 0.05
Distance constraint violations (> 0.1Å)	0
Dihedral angle constraint violations (>3°)	0
Average r.m.s.d. to the mean coordinates [Å]	
Backbone (residues 129-225)	1.58 ± 0.38
Heavy (residues 129-225)	2.40 ± 0.38
Backbone (residues 129-130,144-153,162-163,173-194,200-225)	0.62 ± 0.14
Heavy (residues 129-130,144-153,162-163,173-194,200-225)	1.19 ± 0.11
Ramachandran statistics [%]	
Most favored	90.2
Additionally allowed	8.3
Generously allowed	0.7
Disallowed	0.8

SUPPLEMENTAL REFERENCES

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