Supplementary Materials and Methods

NMR backbone resonance assignment experiments for the full length prion protein

All backbone assignment spectra were recorded on a Bruker Avance DRX-600 spectrometer at 303K. Data were processed and analyzed on Silicon Graphics workstations using Felix 2000 software (kindly donated by Accelrys, San Diego). To assign residues 23-125, the following experiments were recorded: 2D $^{1}H^{15}N$ HSQC, 3D HNCO, 3D HN(CA)CO, 3D HNCACB and 3D CBCA(CO)NH. Assignment was based on linking of the preceding carbonyl (pC') and intra-residue carbonyl shifts (C') because Cα and Cβ shifts are poorly dispersed for the same residue type in this unfolded domain of the protein. To give better resolution, the HNCO and HN(CA)CO were recorded with long ^{15}N and ^{13}C acquisition times, made possible by the slower ^{15}N and ^{13}C T_2 relaxation in this region.

Since the sensitivity of the experiments was not sufficient to collect shift data for spin systems arising from most of the C-terminal domain and an assignment was already available for residues 91-231 [1], assignment was only pursued for residues 21-127. Backbone assignments were arrived at using the simulated annealing program in the 'asstools' suite of programs [2]. The assignment uses energy functions - the linking energy, which describes how well sequential shifts match, and the binding energy, which describes how well the chemical shifts of spin systems match those expected for the residue types to which they are assigned. By default and here, these are weighted 4:1. Because all residues in the N-terminal domain of the protein are unfolded, the Cα and Cβ shifts would be expected to be close to random coil values. Therefore the standard table of Cα and Cβ shifts ranges for different residue types, used by the program, was replaced with a table containing ranges of ±0.5 ppm from the random coil values. The linking energy function was weighted 1:1:4 for Cα, Cβ and C' shifts respectively. Square well energy functions were used throughout. Sequential shift matches were penalized when outside of a 0.1 ppm range for Cα and Cβ shifts and 0.02 ppm range for C' shifts.

Initial inspection of the data revealed that the octapeptide repeat sequences are degenerate in chemical shift and produce only seven highly intense spin systems, one for each

residue in the repeat unit with the exception of the proline, which produces no [N,H] signal. The amino acid sequence used for the backbone assignment therefore included only one octapeptide repeat sequence and the assignment program was set not to assign spin systems to residues 54 and 88 (i.e. the positions were nulled). $^{1}H^{15}N$ HSQC peaks were ranked in order of intensity and after the exclusion of non-backbone peaks and peaks known to arise from the C-terminus, an appropriate number of the most intense spin systems were used for the assignment. Following initial runs of the automated assignment program a number of problematic regions were identified. Residues 21-23 could not be assigned because these spin systems were apparently undetectable, and these positions were therefore nulled. Certain groups of glycine residues (positions 30,46; 54,93; 55,63,71,79,87,94 and 56,64,72,80,88) were found to be completely degenerate in terms of all available chemical shift data. These were nulled in the automated assignment and assigned manually. Duplicate spin systems ascribed to *cis*/*trans* proline isomerism were present around prolines 39, 44, 50, 51 and the octapeptide repeat proline. The minor isomeric form was assigned manually and the minor spin systems excluded from the automated assignment. Residues H96, S97 and H111 were assigned manually. The remaining positions were assigned uniquely in 30/30 cases by the automated assignment program.

NMR sidechain resonance assignment experiments for the full length prion protein

All sidechain assignment spectra were recorded on a Bruker Avance AV-800 spectrometer at 303K, with the exception of the 3D HCCH TOCSY spectrum which was recorded on a Bruker Avance DRX-600 spectrometer at the same temperature. To assign sidechain signals of interest in the aliphatic $^{1}H^{13}C$ HSQC spectrum of the protein, CBCA(CO)NH and HBHA(CO)NH experiments were acquired to obtain Cα and Cβ and Hα and Hβ shift information for assigned backbone spin systems. These experiments allowed assignment of the [Cα,Hα] and [Cβ,Hβ] resonances for all serine and histidine residues from the unfolded domain of the protein. Serine assignments for the folded domain were taken from the assignment of PrP^{91-231}.

A 3D HCCH TOCSY experiment was used to confirm that the two non-histidine signals that disappeared from the aliphatic $^{1}H^{13}C$ HSQC on addition of Cu^{2+} correspond to a single serine spin system. The octapeptide histidine and histidine 96 and 111 Hδ frequencies were assigned using a 2D (HB)CB(CGCD)HD spectrum which correlates Cβ and Hδ frequencies in aromatic residues [3].

1 Hosszu, L. L. P., Jackson, G. S., Trevitt, C. R., Jones, S., Batchelor, M., Bhelt, D., Prodromidou, K., Clarke, A. R., Waltho, J. P. and Collinge, J. (2004) The residue 129 polymorphism in human prion protein does not confer susceptibility to CJD by altering the structure or global stability of PrPC. J. Biol. Chem. **279**, 28515-28521

2 Reed, M. A. C., Hounslow, A. M., Sze, K. H., Barsukov, I. G., Hosszu, L. L. P., Clarke, A. R., Craven, C. J. and Waltho, J. P. (2003) Effects of domain dissection on the folding and stability of the 43 kDa protein PGK probed by NMR. J. Mol. Biol. **330**, 1189-1201

3 Yamazaki, T., Formankay, J. D. and Kay, L. E. (1993) 2-Dimensional Nmr Experiments for Correlating C-13-Beta and H- 1-Delta/Epsilon Chemical-Shifts of Aromatic Residues in C-13- Labeled Proteins Via Scalar Couplings. J. Am. Chem. Soc. **115**, 11054-11055