Supporting Information for "Characterization of β-sheet structure in Ure2p$_{1-89}$ yeast prion fibrils by solid state nuclear magnetic resonance"

I. Determination of isotopic enrichment in Ure2p$_{1-89}$ samples

Isotopic enrichment of selectively labeled Ure2p$_{1-89}$ samples was quantified by mass spectrometry. Aliquots of labeled and unlabeled filaments of about 50 µl (~20 µg peptide) were centrifuged (20,000 rpm, 30 min) to sediment all filaments and redissolved in SDS-sample buffer with 10 M urea, boiled for 2 min, and run on SDS-PAGE gels. After colloidal coomassie staining, the Ure2p$_{1-89}$ band was excised. For site-specific labeled peptides the bands were subjected to in-gel trypsin digestion. LC/MS was performed using a CapLC/QTOF-2 (Waters) using a microscale C-18 column. All ions eluting from the column that were more intense then a certain threshold were fragmented to produce MS2 data. The MS1 data were used as a first assessment of the extent of ^{13}C incorporation. MS2 data were used to check the consistency of the assignment of a given signal to an expected peptide sequence and also to localize the apparent shift in mass compared to a non-labeled peptide. In one case an in-source fragmentation was performed and the quadrupole was used to isolate a fragment for an MS3 fragmentation. An estimate of the percent incorporation was calculated using as a starting point the isotope distribution calculated for an imaginary form of the peptide containing all but the labeled atom to result in a natural isotope distribution not influenced by the labeled atom. The intensities of corresponding peaks in this "starting" distribution and in the experimentally observed isotope distribution were then related to each other with the assumption that only isotope enrichment at the labeled atom is responsible for observed differences. To insure the linearity of peak intensities detector saturation was carefully avoided and the quadrupole was used in a broad resolution setting. In contrast to direct amino acid level analysis this approach is not sensitive to other proteins (both from expression and introduced in handling) present in the sample. Since deamidation reactions also can shift peptide masses by +1 Da, this approach is sensitive to the presence of deamidation reactions if the peptide contains Asn or Gln residues. Although deamidation should similarly be detected in unlabeled control peptides, any influence from deamidation could also be ruled out in most cases by analyzing fragmented peptides. In the case of Leu-^{13}CO/Ala-^{13}CH$_3$-Ure2p$_{1-89}$ the peptide MMNNNGNQVSNLSNALR was used for analysis. Fragmentation could be used to give rise to the y5 ion SNALR, which was clearly shifted by one Dalton in the labeled sample indicating significant incorporation at either leucine or alanine or medium incorporation for both. This y5 ion could also be created through in-source fragmentation in the QTOF-2 making it possible to select it as a parent ion for fragmentation (resulting in MS3 data). The b3 ion (SNA) in the labeled sample was observed to have only minor differences compared to the unlabeled sample and, importantly, the isotope distribution of the b2 ion (SN) was unchanged excluding any influence of deamidation. This indicated low isotope enrichment for alanine and high enrichment for leucine. Fragmentation of the y5 ion gave also rise to an apparent fragment at the mass expected for a putative z2 ion (LR), which has a dramatic difference in isotope distribution between the labeled and unlabeled samples. This is consistent with the presence of a site of high ^{13}C incorporation in this fragment. While the route for formation of the z2 ion is not clear, this observation is internally consistent with the rest of the observations, since the calculated isotope enrichments for alanine and leucine from b3 and z2 ions also predicted correctly the shift of isotope distribution of the y5 ion. For Val-^{13}CO-Ure2p$_{1-}$

$_{89}$ and Arg-^{13}CO-Ure2p$_{1-89}$ the isotopic distribution of the tryptic peptide QVNIGNR was used to estimate isotope enrichment.

For U-^{13}C,^{15}N-Ure2p$_{1-89}$ the excised bands were extracted twice with 0.2 ml 50% (w/v) acetonitrile in 25 mM Tris, pH 8.1, followed by 0.2 ml 100% acetonitrile, and dried in vacuum. The gel pieces were re-hydrated with 0.02 ml 20 μg/ml modified trypsin (Promega) in 25 mM Tris, pH 8.1, and digested overnight at 37°C. Peptides were extracted with 0.1 ml 0.2% (w/v) trifluoroacetic acid, extracts were freed from salts with zip-tip pipette tips (Millipore) and eluted with 3 μl 50 mM alfa-cyano-4-hydroxycinammic acid in 50% (v/v) acetonitrile/0.1% (w/v) TFA directly on the mass-spectrometer sample plate. Peptides were analyzed on an Applied Biosystems Voyager DE-Pro time-of-flight mass spectrometer in the reflectron mode. Internal mass calibration of raw spectra was performed on contaminant trypsin autolysis peaks.

Isotopic enrichment was calculated to be ~31% for $^{13}CH_3$ of alanine and ~92% for ^{13}CO of leucine in Leu-^{13}CO/Ala-$^{13}CH_3$-Ure2p$_{1-89}$, ~75% for ^{13}CO of valine in Val-^{13}CO-Ure2p$_{1-89}$, and ~33% for ^{13}CO of arginine in Arg-^{13}CO-Ure2p$_{1-89}$. The isotopic enrichments of $^{13}CH_3$ of alanine in Leu-^{13}CO/Ala-$^{13}CH_3$-Ure2p$_{1-89}$ and of ^{13}CO of arginine in Arg-^{13}CO-Ure2p$_{1-89}$ are too low to obtain useful NMR data and no further analysis was attempted with these sites/specimens. The isotopic enrichment for U-^{13}C,^{15}N Ure2p$_{1-89}$ was estimated to be greater than 98% from the isotopic distribution of mass peaks.

The scrambling of labels to other sites was assessed by liquid-state ^{13}C NMR spectroscopy of acid-hydrolyzed Ure2p$_{1-89}$ samples. For acid hydrolysis ~4 mg of peptide was dissolved in 1 ml of a 1:1 mixture of HCl and propionic acid, heated to 150°C for 30 minutes in sealed, evacuated ampoules, and dried under a continuous stream of dry nitrogen gas. Hydrolyzed material was then dissolved in ammonium carbonate buffer, filtered through a 0.2 μm filter, and lyophilized. The resulting powder was dissolved in 0.5 ml 10% D_2O (in H_2O) and the pH was adjusted to pH 7.8 with diluted HCl and NaOH. Proton-decoupled 1D ^{13}C NMR spectra were obtained on a Varian Mercury at 300 MHz. Reference spectra were obtained by dissolving the corresponding carboxyl ^{13}C-labeled amino acids (99 % enrichment, Cambridge Isotopes Laboratory) and by taking similar spectra of diluted propionic acid in ammonium carbonate buffer. No scrambling of labels was detected in Leu-^{13}CO/Ala-$^{13}CH_3$-Ure2p$_{1-89}$ and Val-^{13}CO-Ure2p$_{1-89}$ samples (detection threshold is ~5%, limited by signal-to-noise in ^{13}C NMR spectra). The only visible peaks in the spectra could be assigned to the specifically labeled site and to naturally occurring ^{13}C in the residual amounts of propionic acid and carbonate in the solution.

II. HET-$s_{218-289}$ expression and fibril formation

The prion domain of *het-s* of *Podospora anserina* (encoding residues 218-289) was amplified with a C-terminal His_6 tag from pET21a-Hets (kindly provided by Sven Saupe, Bordeaux) by PCR and inserted between the NdeI and HindIII sites of pET21a(+) (Novagen). Uniformly ^{15}N, ^{13}C-labeled HETs(218-289)His_6 was expressed in *E. coli* BL21(DE3) RIL (Clonetech) in the same medium as described for uniformly labeled $Ure2p^{1-89}$ (Methods). After growth to OD_{550} = 0.6 without label, cells were collected and resuspended in media with 2g/l of $^{15}NH_4Cl$ and 0.5 g/l of glucose-U-^{13}C. After 15 min, 1 mM IPTG and 4.5 g/l glucose-U-^{13}C were added and growth continued for 12 hours. Cells were suspended in 0.1 M Tris Cl pH 8.0, 150 mM NaCl, lysed using an Aminco French Press, and inclusion bodies collected by centrifugation at 30,000 rpm for 20 min in a 45Ti rotor at 4C. The pellet was dissolved in 8M guanidine HCl, 0.1 M Tris Cl pH 8.0, 150 mM NaCl, cleared by 20 min centrifugation at 20,000 rpm in the 45Ti rotor, and the His_6-tagged protein was purified by a Talon column and desalting PD10 column as described (1,2). The ~95% homogeneous product (~20 mg/liter of culture) was neutralized and allowed to form filaments at 4C. After ~5 days a gel had formed. Filaments were pelleted at 7,000xg, washed four times with water and, in most cases, lyophilized.

1. Dos Reis, S. et al. The HET-s prion protein of the filamentous fungus Podospora anserina aggregates in vitro into amyloid-like fibrils. *J Biol Chem* **277**, 5703-6 (2002).
2. Balguerie, A. et al. Domain organization and structure-function relationship of the HET-s prion protein of *Podospora anserina*. *Embo J* **22**, 2071 - 2081 (2003).

III. fpRFDR-CT data for selectively labeled Ure2p$_{1\text{-}89}$ fibrils, before correction for natural-abundance ^{13}C NMR signals