Supplementary Figures

Figure S1: Monoexponential deuterium exchange rates in HET-s(218-289) fibrils. Peak volumes of representative residues are plotted versus the logarithm of the exchange time t_{ex}. Data are shown for V223 (red), T233 (violet), E234 (green), V245 (yellow), E265 (cyan) and Y281 (blue). Smooth solid lines represent fits of the data to a monoexponential equation.

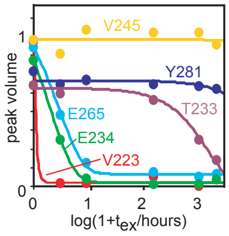
Figure S2: Deuterium exchange rates of residues 218-289 in full length HET-s. Observed exchange rates k_{ex} / h^{-1} are plotted against the amino acid sequence of residues 218-289 of HET-s. Exchange time points were collected between 20 minutes and 3696 hours. The measurable upper limit was 15 h^{-1}. Blue arrows indicate the location of β-strands determined for the fragment HET-s(218-289).

Figure S3: Sequence comparison of the β1β2 (226-246) and β3β4 (262-282) regions of the HET-s prion forming domain

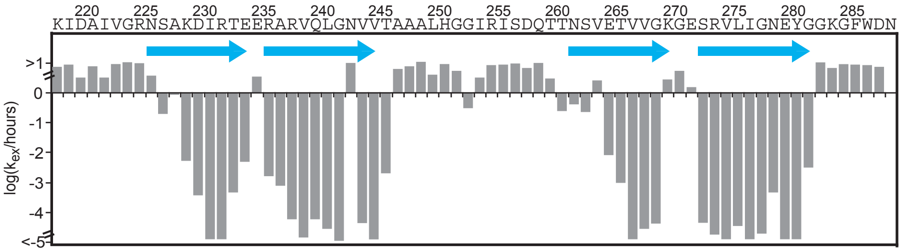
Amino acid sequence (**a**) and nucleotide sequence alignments (**b**) were performed with the ClustalW algorythm. Identical residues are boxed in black and similar residues are boxed in grey.

Figure S4: Electron micrographs of aggregates of recombinant wild-type and mutant HET-s proteins. Negatively stained aggregates of wild-type and mutant HET-s 226P, 246P and 266P protein are shown. (scale bar is 100 nm). Mutant HET-s 226P, 246P and 266P proteins were expressed in *E. coli* and purified. The aggregation of HET-s 226P and 266P was very inefficient but small amounts of fibrillar material could be detected by electron microscopy.

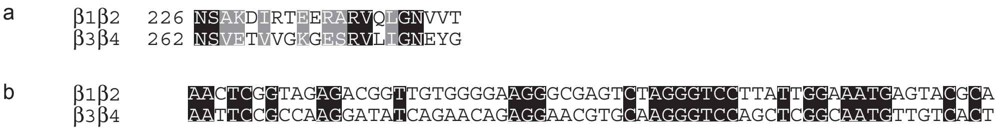
Figure S5: One-dimensional traces from the 2D spectra shown in Figure 1c,d. Trace a is from the up-down tangential sweep at 40 kHz MAS frequency, the Cβ/C’ crosspeak is positive because this is a two step transfer, trace b is from the down-up sweep and the Cα/C’peak is a one-step transfer which leads to negative peaks. Traces c and d are from the 25 kHz spectrum.



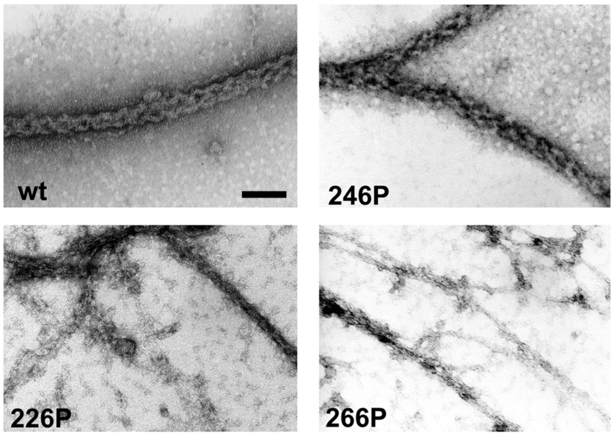
Supplementary Figure S1



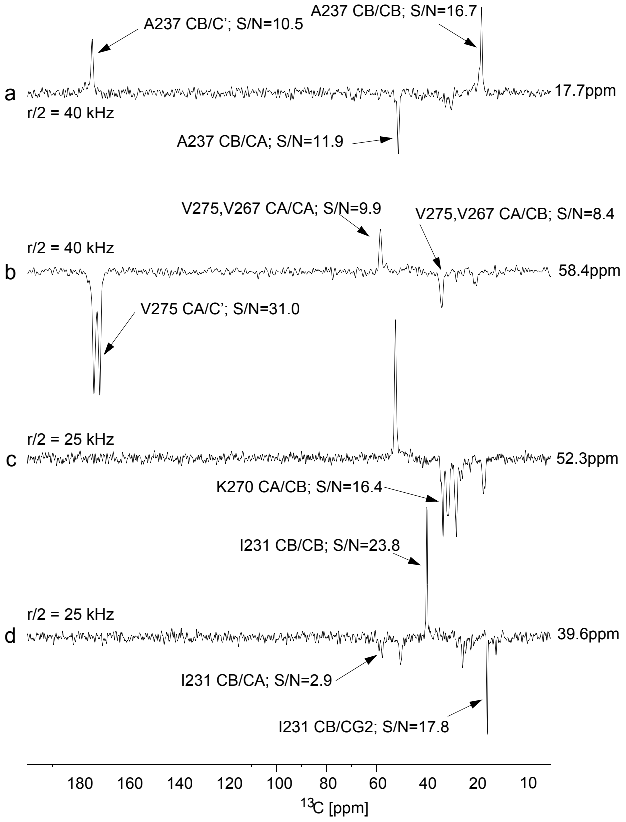
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5

Supplementary Table 1 **Activity of *het-s* mutants in heterokaryon incompatibility and [Het-s] infectivity assays**

HET-s mutation	positives in cell death reaction	positives in infectivity assay	positives in infectivity assay after subculturing
vector	0 (0/24)	2 (1/48)	2 (1/48)
wt	81 (144/177)	100 (40/40)	100 (24/24)
220 P	95 (45/47)	100 (32/32)	95 (20/21)
226 P	5 (3/64)	0 (0/32)	0 (0/24)
230 P	4 (2/47)	8 (2/24)	0 (0/24)
235 P	9 (8/93)	38 (12/32)	33 (8/24)
240 P	10 (5/48)	60 (19/32)	0 (0/11)
246 P	87 (60/69)	100 (16/16)	88 (7/8)
253 P	79 (37/47)	100 (32/32)	100 (12/12)
260 P	92 (74/80)	100 (32/32)	100 (5/5)
266 P	12 (8/64)	22 (7/32)	48 (11/23)
270 P	5 (3/55)	41 (13/32)	8 (2/24)
276 P	5 (3/64)	33 (16/48)	8 (2/24)
280 P	10 (5/48)	0 (0/24)	0 (0/24)
284 P	83 (41/49)	100 (16/16)	50 (12/24)
∆225-230	0 (0/48)	0 (0/32)	n.d.
∆244-248	2 (1/46)	0 (0/32)	n.d.
∆251-253	82 (37/45)	100 (32/32)	75 (18/24)
∆253-255	70 (17/24)	100 (16/16)	n.d.
∆252-256	53 (18/34)	50 (5/10)	48 (11/23)
∆248-256	0 (0/24)	0 (0/24)	0 (0/48)
∆258-262	0 (0/48)	0 (0/16)	4 (1/24)
∆276-279	0 (0/24)	0 (0/24)	n.d.

Deletion and proline-scanning *het-s* mutants are listed. The first column gives the percentage of transformants producing a cell death reaction when confronted with a *het-S* tester strain. The number of positives over the total number of tested transformants is given in parentheses. The second column and third column gives the percentage of transformants that were able to infect a wild-type [Het-s*] prion-free recipient strain. In the experiments reported in the second column, transformants were tested immediately after contact with a wild-type [Het-s] prion-infected donor strain. In the experiments reported in the third column, the transformants were first subcultured for 3 days after their initial contact with the wild-type [Het-s] donor strain. (n.d., not determined). Spontaneous appearance of [Het-s] is in the range of 2 % in the tested conditions.

Supplementary table 2 **Infectivity of mutant recombinant HET-s 226P, 246P and 266P proteins in biolistic assay**

protein	[Het-s]-positives
buffer	0 (0/32)
wt	100 % (64/64)
226 P	25% (9/36)
246 P	97% (39/40)
266 P	67% (20/30)

Percentage of [Het-s]-positive transformants obtained after biolistic introduction of the listed proteins is given together with the number of positives over the total number of tested strains.