

Supplementary Information

The most infectious prion protein particles

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1. Supplementary Methods

Analysis of filtered converting activity

Samples of purified, PK-digested 263K PrP-res (2 mg/mL) were pelleted (20,800 x g, 20 min, 4°C), and resuspended in an equal volume of 0.25 M Tris pH 7.0. Aliquots (0.5-7.5 uL) were diluted to 0.1 mg/mL in the same buffer containing various detergents, without sonication. Samples were subjected to a 5 min freeze/thaw cycle, incubated at 37°C for one hour, and filtered by centrifugation (14,000 x g, 3 min or 500 x g, 20 min) at 25 °C through 0.2 µm or 300 kDa (~30 nm) Nanosep centrifugal devices. Filtrates of the material were analyzed by solid-phase conversion assay, and the formation of small particles that retained converting activity was assessed by the levels of converting activity in filtrates, which were expressed as a percentage of the level of converting activity detected in untreated, unfiltered PrP-res.

Suspension-based PrP conversion

Samples (20 µL) of fractionated PrP-res were analyzed in suspension-based conversion reactions (40 µL) containing 40,000 CPM ³⁵S-PrP-sen under the same conditions used for solid-phase conversion reactions except for the presence of residual SUS (0.05%) and Tris (10 mM) derived from the samples. PK (20 µg/mL) digestion, SDS-PAGE, and conversion efficiency calculations were performed as described previously¹.

Scrapie infectivity titrations

Groups of Syrian golden hamsters were inoculated intracranially (50 μ L/animal) with successive 10-fold dilutions of either 1% brain homogenate or purified PrP-res. Dilutions were made in physiological phosphate buffer containing 2% fetal bovine serum.

Homogenates ($\sim 10^8$ LD₅₀/50 μ L) were derived from the brains of hamsters clinically-affected with the 263K strain of hamster scrapie. LD₅₀ values were determined as previously described², and the incubation period of disease was defined as the number of days from inoculation to euthanization at end-stage clinical disease (recumbent animals).

Determination of specific infectivity, specific converting activity, and statistical analyses

Levels of infectivity (LD₅₀) were first determined from the incubation period of disease for each animal by using the equation fit to the data in Supplementary Figure S4b:

$$LD_{50} = 5.21 \times 10^{51} * T^{-24.097},$$

where T is the incubation period of disease in days.

The mean and standard error (SE) for LD₅₀ values, converting activities, and PrP amounts were then determined for each fraction.

The levels of scrapie infectivity and PrP converting activity with respect to PrP amount (specific infectivity and specific converting activity respectively), are defined as:

Specific infectivity = infectivity/PrP amount

Specific converting activity = converting activity/PrP amount

The standard errors of specific infectivity and specific converting activity were derived by using a Taylor series expansion. Note that specific converting activity and specific infectivity are estimated by the ratio of two averages: the average converting activity or infectivity (Y) divided by the average PrP amount (X). The standard error of specific converting activity or specific infectivity (Y/X) can be approximated by:

$$\sqrt{\frac{SE(Y)^2}{E(X)^2} + \frac{E[Y]^2 * SE(X)^2}{E[X]^4}},$$

where E[X] and SE(X) are the mean and the standard error of PrP amount, and E[Y] and SE(Y) are the mean and the standard error of converting activity or infectivity.

2. Filtration of converting activity in SDS-treated samples of PrP-res.

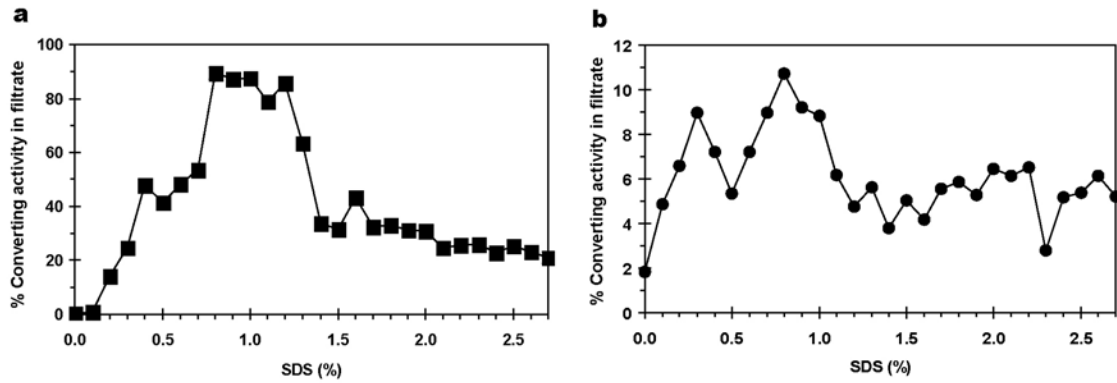


Figure S1 Samples of PrP-res were treated (1 hr, 37° C) with SDS at concentrations from 0 to 2.7% without sonication, passed through either 0.2 µm (a) or 300 kDa (b) filters, and the filtrate was analyzed for *in vitro* PrP-converting activity by solid-phase conversion assay.

3. 300-kDa filtration of converting activity in alkyl sulfate-treated samples of PrP-res.

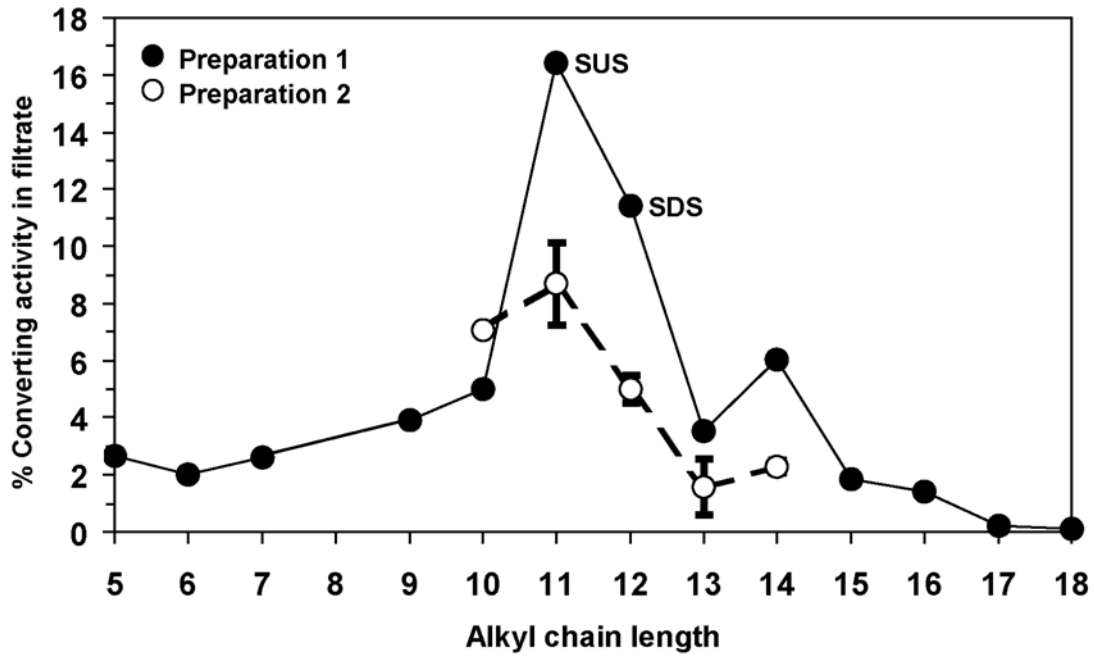


Figure S2 Samples of PrP-res were treated (1 hr, 37° C) with 1% alkyl sulfate solutions without sonication, passed through a 300-kDa filter, and the filtrate was analyzed for *in vitro* PrP-converting activity by solid-phase conversion assay. Values are shown for two different purified stocks of PrP-res, and for preparation #2 represent mean \pm range (n = 2). The samples treated with SUS and SDS are indicated for preparation 1.

4. Suspension-based conversion assay of fractionated PrP-res.

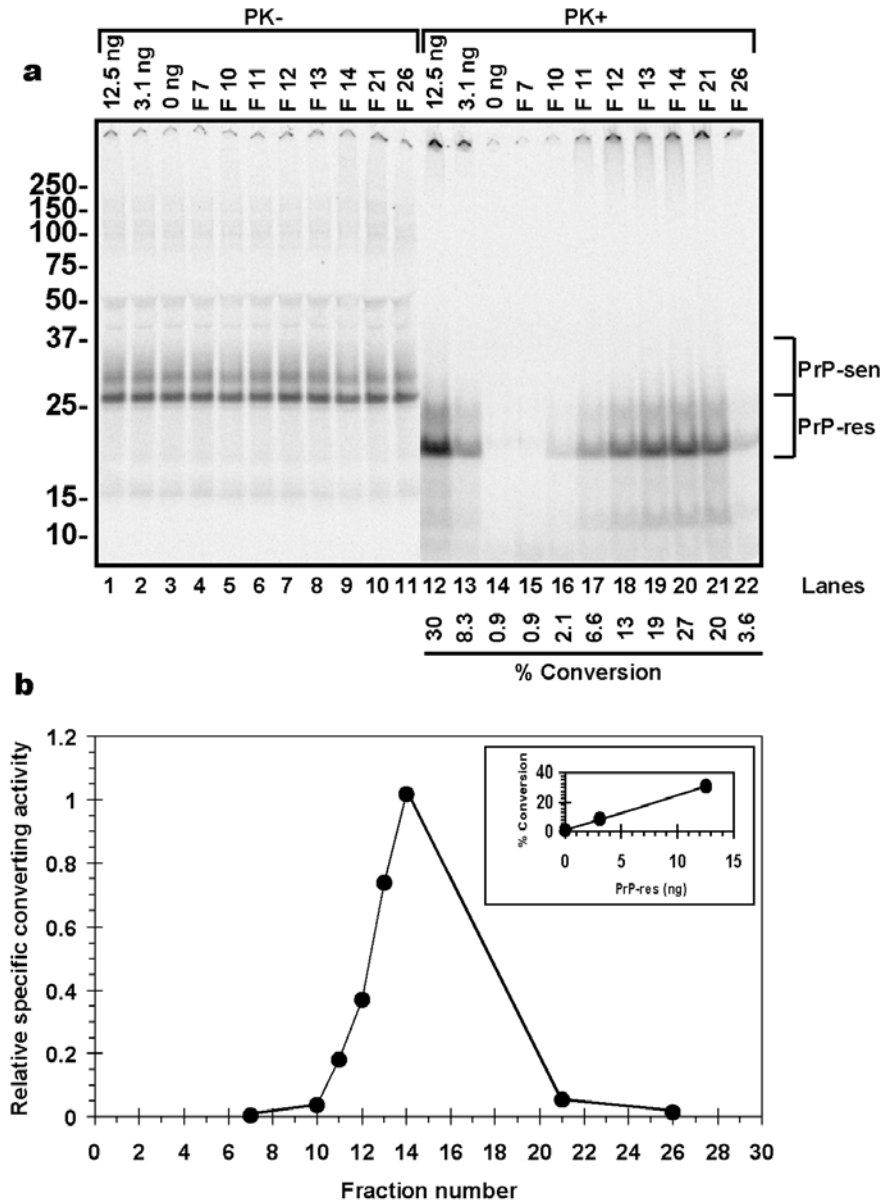


Figure S3 a, autoradiogram of products from suspension-based conversion assay of select FIFFF fractions. Lanes 1-11 represent 1/10 of the ^{35}S -PrP-sen applied to the conversion reactions, and lanes 12-22 represent the remaining 9/10 of the conversion reactions, which were digested with PK. Control reactions with PrP-res (lanes 1-3 and 12-14) were analyzed to relate amounts of PrP-res to percent conversion. Fraction

numbers or amounts of PrP-res (ng) are shown at the top, and the percent conversion (bottom) was calculated using the ^{35}S -PrP bands indicated to the right. Molecular weight standards (kDa) are shown on the left. **b**, Calculated specific converting activity. The relationship between the amount of input PrP-res, and the percent conversion was linear up to at least 12.5 ng PrP-res (inset). The presence of the peak of specific converting activity at fraction 14 was consistent with a slight shift in the elution profile of the PrP-res in this individual analysis and did not represent a notable change in the M_w or size of the particles found at the corresponding peak in solid-phase conversion analyses.

5. Titration of scrapie infectivity.

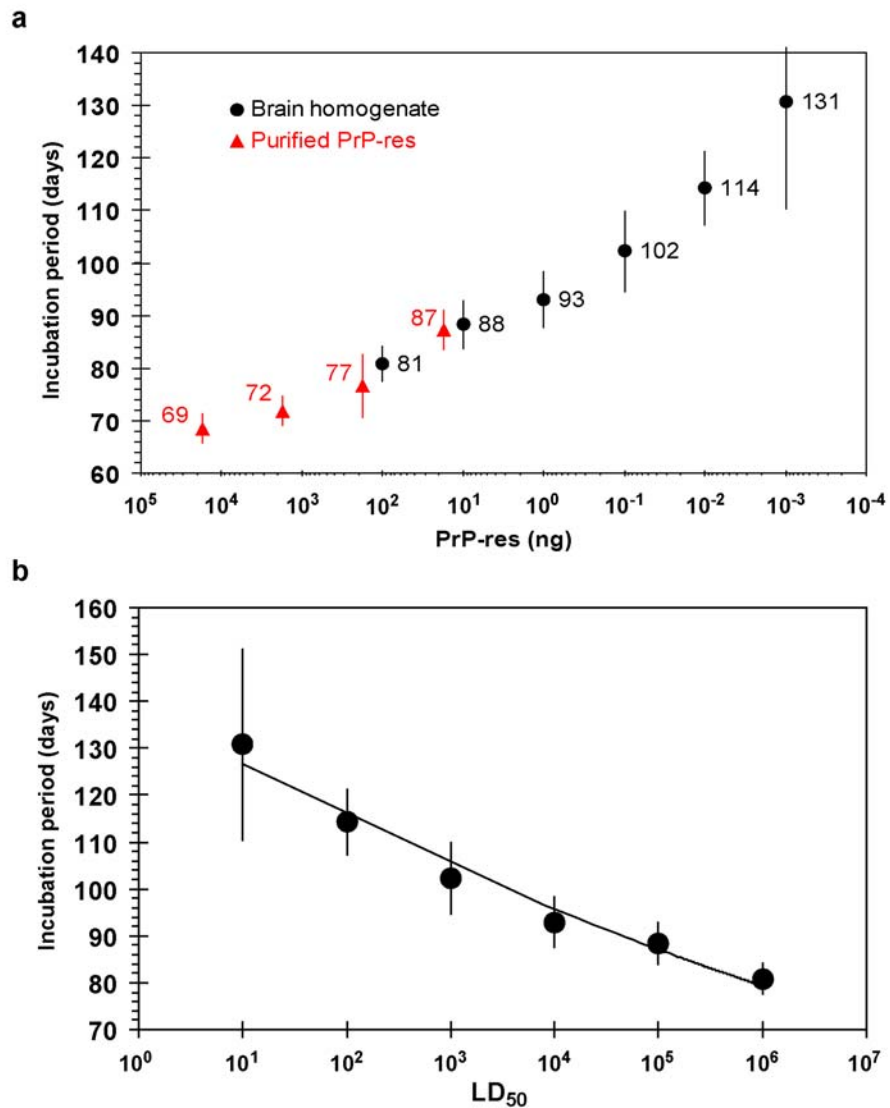


Figure S4 Incubation period of scrapie disease as a function of (a) PrP-res derived from either scrapie infected brain homogenates (n ranged from 18 to 28 animals for each dilution) or purified PrP-res (n = 6 for each dilution), or (b) LD₅₀ (n ranged from 18 to 28 animals for each dilution). Mean incubation periods are indicated by numbers in the plot, error bars represent ± s.d.

6. PAGE analyses of SUS-treated PrP-res.

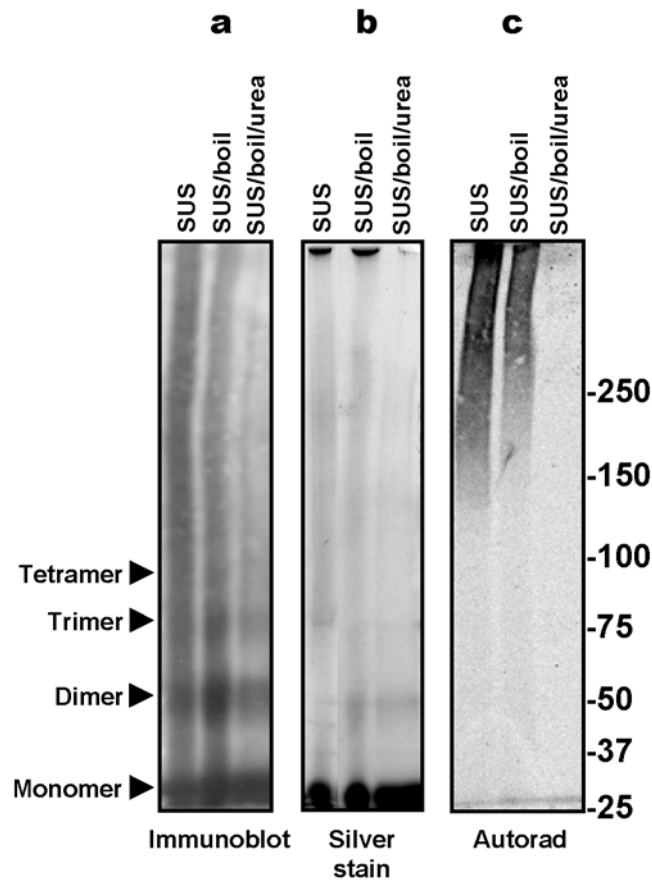


Figure S5 Samples of purified PrP-res were subjected to the SUS disaggregation procedure described in the methods section of the main text, with the addition of 10 min boiling alone, or boiling in urea sample buffer (62.5 mM Tris pH 6.8, 5% glycerol, 5% SDS, 0.02% bromophenol blue, 3 mM EDTA, 4 M urea), separated by PAGE on 3-8% Tris-Acetate gels, and analyzed by either (a) immunoblotting, (b) silver stain, or (c) solid-phase conversion. Note that the lower percentage acrylamide gels used in this figure cannot resolve individual PrP glycoforms, while the higher percentage acrylamide gels used in Fig 2, panels c and d can. PrP oligomers are indicated on the left, and molecular weight standards (kDa) are shown on the right.

7. Denaturing PAGE analyses of fractionated PrP-res.

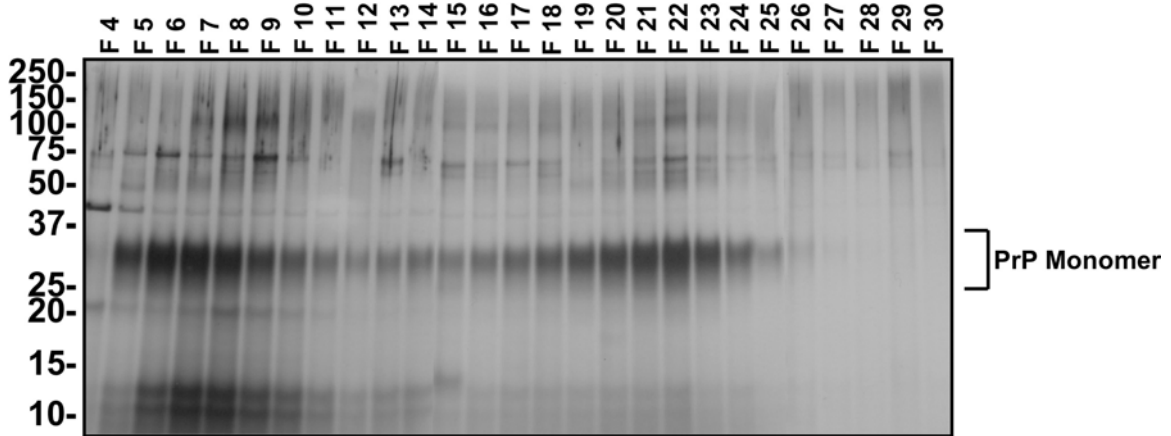


Figure S6 Samples were removed from FIFFF fractions, boiled 10 min in urea sample buffer, subjected to PAGE on 10% Bis-Tris gels, and analyzed by silver stain. Fraction numbers are shown at the top, molecular weight standards (kDa) are shown on the left, and the location of PrP monomer is indicated on the right.

8. Supplementary Discussion

Estimation of infectivity per particle

From our FIFFF data, we can estimate infectivity per particle, rather than per unit PrP. Assuming that most of each particle is PrP (which may not be true for all of the fractions), such calculations suggest that the infectivity per particle values were similar throughout the most infectious FIFFF fractions (10-23 in figure 1), with the smaller non-fibrillar particles (e.g. fraction 12) having values slightly higher, but within 2-fold, of those of large fibrils (e.g. fraction 23) (data not shown). Thus, as long as infectious particles are above a minimum size, particle concentration is a key parameter in determining scrapie infectivity titer.

PrP content of PrP-res aggregates

Based on protein assays and ultra-microbalance measurements, $47\pm 9\%$ of the vacuum-dried weight of the washed, unfractionated SUS-treated PrP-res particles was protein (data not shown), and, according to semi-quantitative western blots (data not shown), $>87\%$ of the protein was PrP. Adjusting for the glycan and glycosphosphatidylinositol content ($\sim 25\%$) of PK-treated PrP molecules, we estimate that at least 54% of the mass was attributable to PrP molecules. In addition, the silver-stained gel in Supplemental Fig. S6 shows that the vast majority of protein in all of the FIFFF fractions was PrP. However, it is unclear how much of the non-PrP mass of the aggregates is tightly bound SUS versus other molecules (e.g. polysaccharide³, glycosaminoglycans⁴, lipids⁵), or what proportion of the most infectious particles is PrP. We have not been able to separate the unidentified silver-stained molecules migrating below the 15-kDa marker from the FIFFF

fractions with PrP and infectivity (Supplemental Fig. S6). These proteins do not react with our anti-PrP antibodies and, so far, have defied identification by MALDI-mass spectrometry of proteolytic fragments (data not shown). Whether these or other molecules are essential components of the most infectious units remains to be determined. Our demonstration of infectivity in particles of a few hundred kDa establishes this mass as the upper limit for any individual molecular component of infectivity, especially if one is to allow for the presence of PrP molecules as at least part of the minimal particle mass.

9. Supplementary References

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