Recombinant ovine prion protein can be mutated at position 136 to improve its efficacy as an inhibitor of prion propagation

Katarzyna Kopycka[1], Ben C. Maddison[2] and Kevin C Gough[1*#]

[1]School of Veterinary Medicine and Science, The University of Nottingham, College Rd., Sutton Bonington, Loughborough, Leicestershire, LE12 5RD. UK.

[2] ADAS Biotechnology, Unit 27, Beeston Business Park, Technology Drive, Beeston, Nottinghamshire, NG9 1LA, UK.

* Corresponding authors

[#] Kevin C. Gough http://orcid.org/0000- 0001-5211-2088

Further supporting data:

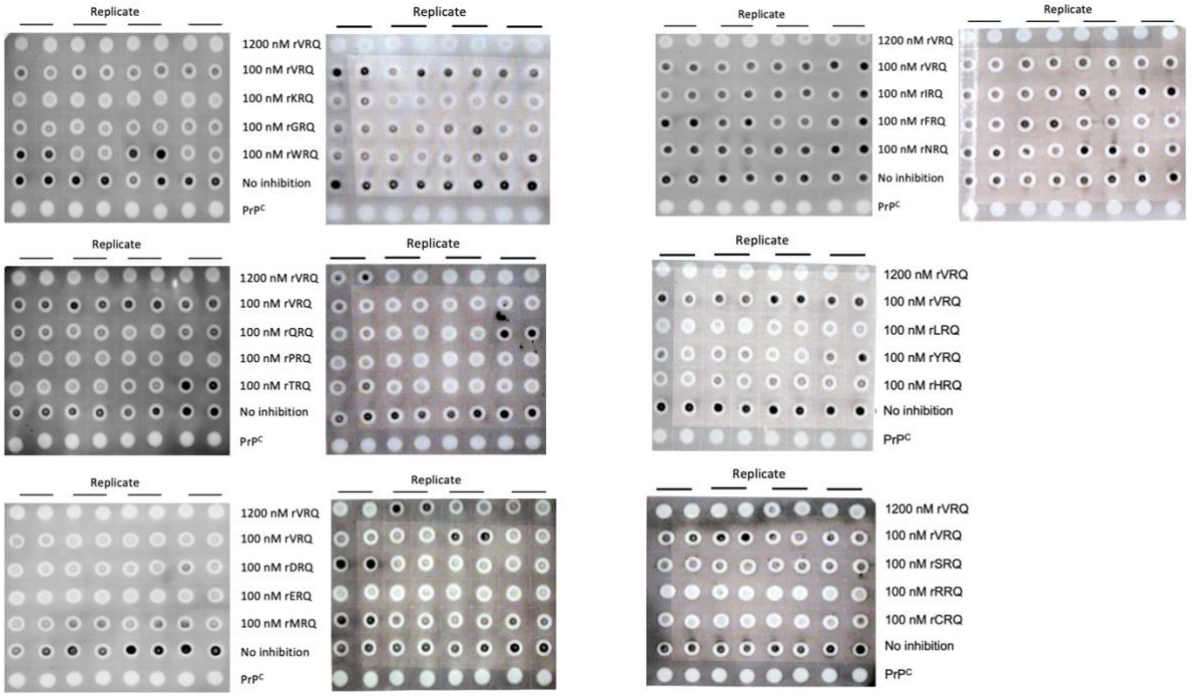

Figure S1. Inhibition of scrapie prion replication with 136 variants of rPrP. PMCA amplification of scrapie VRQ/ARQ prion in a VRQ/VRQ substrate was carried out in the absence or presence of a rPrP as indicated. Each sample was analysed in 8 replicate PMCA amplifications and 4 replicates were analysed (in duplicate) on each of two dot blots. Dot blots were analysed by densitometry and the PrP^{Sc} signals for each duplicate analysis were averaged. Then the signal above the blot background (1200 nM rVRQ inhibition) for each sample were expressed as a percentage of the signal for the no inhibition control. Averages of the 8 PMCA replicates were determined with SD. PrP^{C} (brain PMCA substrate) was used as a PK-digestion control, inhibition with 100 nM rVRQ was carried out in each experiment as a known inhibitor control.

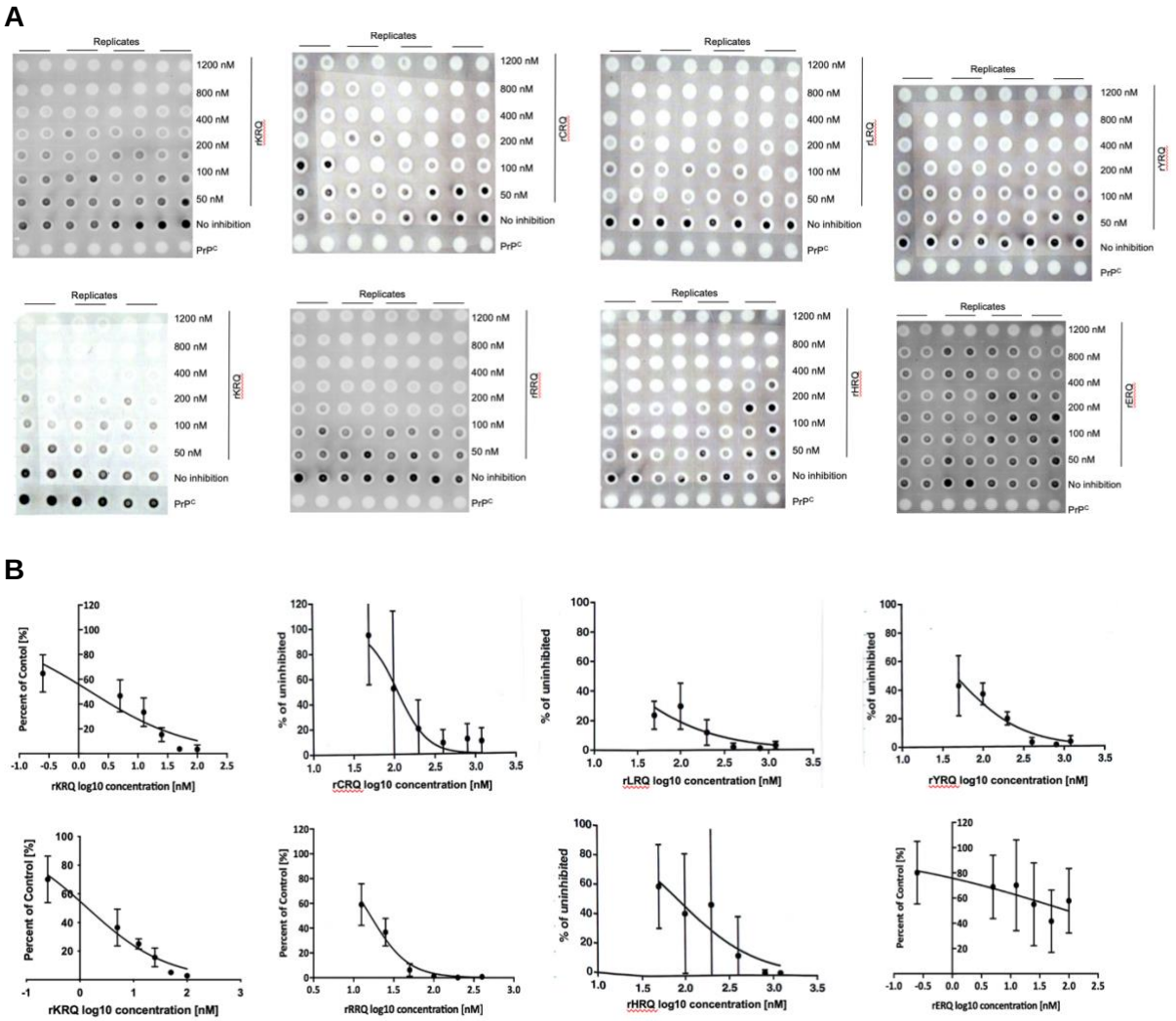

Figure S2. IC50 value determination for rPrP variants at position 136 inhibiting ovine scrapie replication. IC50 values were determined in the PMCA model of prion replication by measuring the level of inhibition for rPrP over 6 concentrations (as indicated for each rPrP). PMCA reactions were carried out in 3 or 4 replicates and each sample analysed in duplicate on dot blots (A). Dot blots were analysed by densitometry and the PrP^{Sc} signal above background (1200 nM rVRQ inhibition) were averaged for the duplicate analyses. The mean of replicate PMCA reactions with SD were plotted to calculate IC50 values (B).

Figure S3. Prion replication inhibition with peptides of rKRQ, rVRQ, rRRQ and rPRQ spanning residue 136. PMCA amplification of scrapie VRQ/ARQ prion (PG1361/05) in a VRQ/VRQ substrate was carried out in the absence or presence of a rPrP peptides (all in triplicate, OvV, OvP, OvR and OvK are peptides from VRQ, PRQ, RRQ and KRQ, respectively). Product was analysed by dot blot (each sample side by side in duplicate). Peptides were composed of amino acid residues 122-139 (added to 50 µM) or 112-144 (usually added to 1 µM,). For all peptides covering residues 112-144 the no inhibition control was carried out in the equivalent dilution of the peptide carrier solvent, 80% (v/v) acetonitrile (N.B. the solvent present with 50 µM peptide inhibited amplification as seen for OvR*). For OvV122-139 the no inhibition control was also carried out in the equivalent dilution of the peptide carrier solvent, 18% (v/v) acetonitrile + 2% (v/v) formic acid. All other no inhibition controls were in PBS as the peptides were dissolved in water. Analysis of dot blot images by densitometry measured the PrP^{Sc} signal compared to corresponding the no inhibition control. Data was analysed using one-way ANOVA and the addition of peptide was not significantly

different to the corresponding no inhibition control for any peptide inhibitor. PrP^C, brain PMCA substrate used as a PK-digestion control.

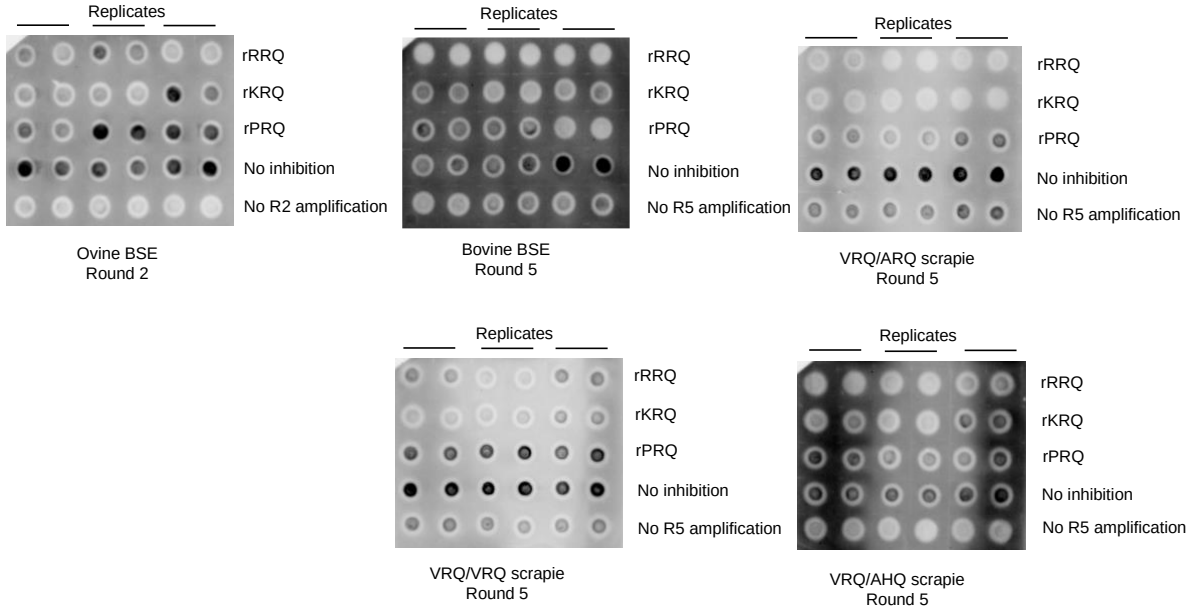

Figure S4. Inhibition of the replication of ruminant prion strains/isolates with 136 variants of rPrP. rRRQ, rPRQ and rKRQ were added (as indicated) into each round of PMCA at 50 nM. PMCA amplification in the absence of any rPrP was carried out as a no inhibition control for comparison. Three replicate PMCA reactions were carried out and each analysed in duplicate by dot blot. Ovine BSE genotype ARQ/ARQ was amplified in 2 rounds using alternating ARQ/ARQ and AHQ/AHQ substrates. Bovine BSE was amplified in bovine brain substrate, and ovine scrapie isolates with VRQ/ARQ, VRQ/VRQ and VRQ/AHQ genotypes amplified in ovine VRQ/VRQ brain substrate; all amplified over 5 rounds and analysed on the same blot with their corresponding no inhibition control and background signal (no amplification in the final round of sPMCA). rRRQ consistently showed high level of inhibition of prion replication compared to the other inhibitors; it demostrated 77%, 98, 99, 90 and 82% inhibition for ovine BSE, bovine BSE, VRQ/ARQ, VRQ/VRQ and VRQ/AHQ scrapie, respectively. rKRQ inhibited these strains by 61%, 81%, 100%, 97% and 57%, respectively; and rPRQ by 17%, 68%, 80%, 40% and 46%, respectively.

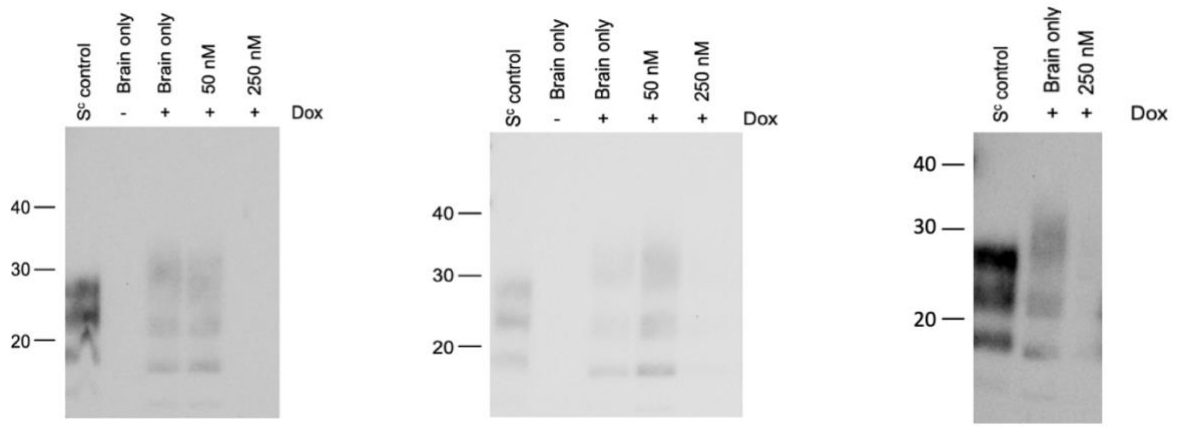

Figure S5. Inhibition of scrapie infection of cells with rRRQ. Rov9 cells expressing ovine VRQ/VRQ PrP^{C} (+ Dox) were inoculated with SSBP/1 scrapie brain homogenate with or without the presence of rRRQ (at 50 or 250 nM, as indicated). After a single passage, cell lysate containing 500 µg of total protein was analysed for PrP^{Sc} levels by western blot. Cells without induction of VRQ/VRQ expression (- Dox, brain only) were included in the first two repeats to demonstrate no PrP^{Sc} inoculum was detected in the analysed samples. Uninhibited cell infections were carried out in the absence of any rPrP (+ Dox, brain only). The experiment was repeated twice with inhibitor at 50 nM and three times with inhibitor at 250 nM, and western blots are shown. SSBP/1 brain (S^{C} control) was digested with 100 µg/ml PK and the equivalent of 7.5 µl of 10% (w/v) brain was analysed as a blotting control. Protein molecular weight markers are indicated (kDa). Densitometry analysis of PrP^{Sc} signals were calculated for each of the triplicate experiments with inhibitor at 250 nM and uninhibited control. Unpaired Student's T-test showed significant differences between 250 nM rRRQ and the no rPrP control (p=0.02).

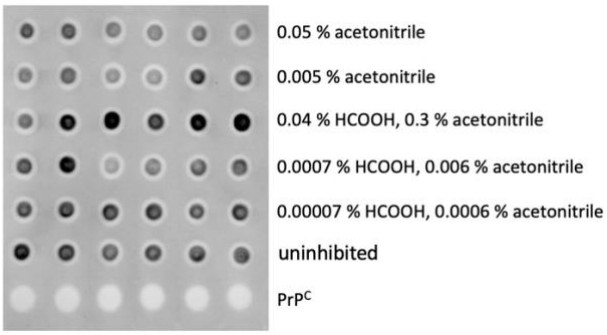

Figure S6. The effects of peptide carrier solvents on prion amplification. For the analysis of PrP peptides as inhibitors, it was first established whether the carrier solvent had any effects on PMCA. The indicated concentrations of solvent were added into PMCA reactions amplifying ARQ/VRQ PG1361/05. After 1 round of PMCA, 2.5 µl of PK digested products were analysed by dot blot in duplicate. PrP^{C}, negative brain homogenate to test PK digestion efficiency. Acetonitrile at 0.05% and 0.005% (v/v) are the concentrations present when adding 1 µM and 100 nM peptide to PMCA reactions, respectively. Both concentrations of solvent produced a significant reduction in a prion amplification compared to the uninhibited control (in PBS). The presence of 0.04% (v/v) HCOOH/0.3% (v/v) acetonitrile, 0.0007% (v/v) HCOOH/0.006% (v/v) acetonitrile and 0.00007% (v/v) HCOOH/0.0006% (v/v) acetonitrile are present when adding 50 µM, 1 µM and 100 nM of peptide, respectively. None of these solvent concentrations had a significant effect on prion amplification. A one-way ANOVA with Dunnett's multiple comparison test was carried out on the densitometry data.