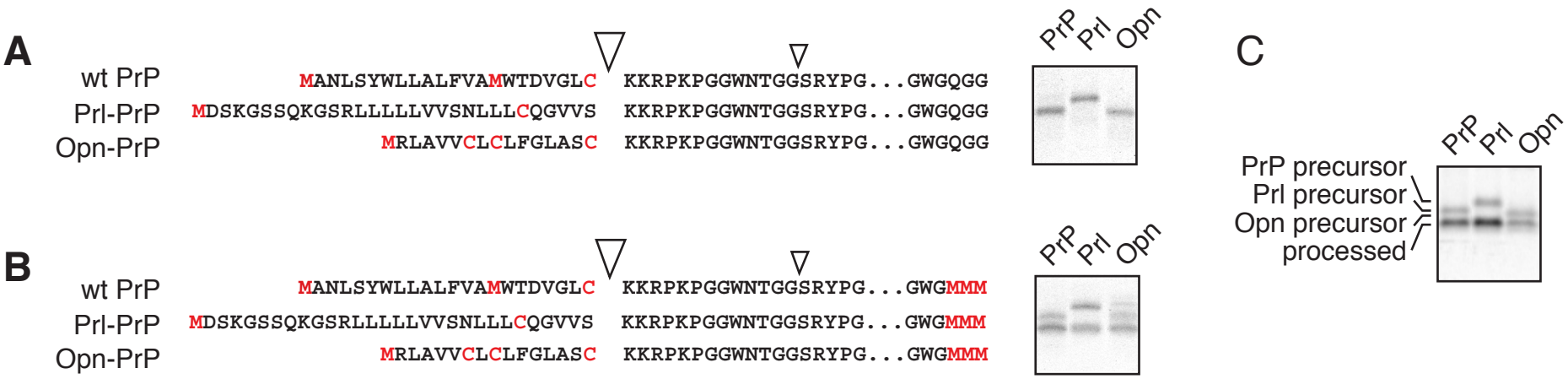

Figure S4 - Analysis of the signal sequence cleavage sites of PrP, Opn-PrP, and Prl-PrP.

(A) Wild type PrP, Opn-PrP, and Prl-PrP were truncated at position 71 of the mature domain and synthesized in vitro (using rabbit reticulocyte lysate and ER-derived microsomal membranes from canine pancreas) using ^{35}S-methionine and ^{35}S-cysteine (exactly as in Kim and Hegde, 2002). On the left are the sequences of the three constructs, with the positions of the methionines and cysteines indicated in red. The predicted site of signal sequence cleavage is indicated by the large arrowhead. Note that the ONLY methionines and cysteines in each truncated construct are located in the signal sequence. On the right, note that only one radiolabeled product is observed, indicating that the signal sequence cleaved product does not contain any methionines or cysteines. Thus, cleavage must occur at a site after the last cysteine of each construct. That cleavage is indeed occurring can be demonstrated by parallel analysis **(panel B)** of constructs in which methionines are introduced into the mature domain (as indicated in red). Here, both signal uncleaved and signal cleaved products can be observed. Note that the signal cleaved products all migrate at the same position, while differences in migration can be seen for the uncleaved products. The difference in length between the Prl and PrP signal sequences is 8 residues (which can be clearly resolved), illustrating the resolution of these Tris-tricine gels in this size range. This means that the signal cleaved products for the three constructs (which migrate identically) cannot differ in size by more than 8 residues. Thus, cleavage at any alternative site for Opn-PrP and Prl-PrP must occur after the last cysteine in the signal, and at a site less than 8 residues from the predicted cleavage site. However, a comprehensive analysis of over 160 signal sequences (von Heijne, 1986, *Nuc. Acid Res.*, 14:4683-4690) revealed that Proline is *never* found at the +1 or -3 positions (relative to the cleavage site), and that Lysine, Asparagine, Arginine, and Tryptophan are *never* found at positions -1 or -3. With these constraints, the only feasible alternative cleavage site downstream of the predicted site is 13 residues away (indicated by the small arrowhead). If Prl-PrP or Opn-PrP were being cleaved here, a size difference would have been seen; however, no difference is seen in the signal cleaved product under conditions where the size differences between the precursors of PrP, Prl-PrP, and Opn-PrP can be resolved **(panel C)**. This demonstrates that in all three cases, cleavage is very likely to be occurring at the predicted site (indicated with the large arrow head).