Chandramowlishwaran et al Nucleation of a yeast prion by mammalian proteins



Anti-Sup35M

Figure S4. Accumulation of chimeric proteins in yeast. (A) HA-tagged Sup35N-PrP90-230 protein is accumulated at higher levels, compared to HA-tagged Sup35N protein, as detected by SDS-PAGE and Western blotting, followed by reaction to anti-HA antibody. The upper band in the right lane corresponds to the dimer, presumably formed via disulfide bonds, as it is sensitive to β-mercaptoethanol (data not shown). (B) Levels of Sup35N-PrP90-230 and Sup35N-PrP120-230 proteins are similar to each other, while Sup35N-PrP90-159 and Sup35N-PrP90-171 proteins are accumulated at lower levels, as detected by SDS-PAGE and Western blotting with anti-Sup35N antibody. Similar levels of Sup35N-PrP90-230 and Sup35N-PrP120-230 proteins are also confirmed by using the anti-PrP (4H11) antibody (data not shown). (C) Sup35N-AB1-40 and Sup35N-AB1-42 proteins are accumulated at similar levels, as detected by SDS-PAGE and Western blotting with anti-Aß (6E10) antibody. (D) Sup35N-AB1-42 and Sup35N-AB1-42\*\*\* (triple F19S, F20S, I31P substitution) proteins are accumulated at similar levels, as detected by SDS-PAGE and Western blotting with anti-AB (6E10) antibody. (E) Comparison of the levels of Sup35NM, Sup35NM-A
<sup>β</sup>1-40, and Sup35NM-A
<sup>β</sup>1-42 constructs as detected by SDS-PAGE and Western blotting with anti-Sup35M antibody. Chimeric proteins are accumulated at similar levels, which are lower than the level of accumulation of Sup35NM. In all cases, amounts of total protein loaded were normalized by the Bradford assay and/or Coomassie staining. On panel E, the upper band, corresponding to full-length Sup35 protein also serves as a loading control. On all panels, "+Cu" refers to cultures growing in the presence of additional 100 µM CuSO<sub>4</sub>. Positions of molecular weight markers are indicated.