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Finally, to examine the potential role of clathrin heavy chain in the uptake of Ure2 aggregates, we generated several DKOR lines that express either wild-type or mutant forms of clathrin heavy chain using retrovirus-based transfection. These lines were then assessed for their uptake of Ure2 aggregates using immunofluorescence and measuring the degree of growth inhibition. DKOR cells that expressed wild-type clathrin heavy chain could take up native Ure2, protofibrils and fibrils, and showed no marked decrease in the uptake compared with clathrin-negative cells (Fig. 6A). However, DKOR cells expressing mutant (GFP-tagged) clathrin heavy chain, which could not assemble clathrin coats, could not take up native Ure2, protofibrils and fibrils, nor did they show any marked decrease in the uptake compared with clathrin-negative cells (Fig. 6B and D). In summary, we found that uptake of Ure2 aggregates into cells is mediated by clathrin-dependent endocytosis. This was most marked in the case of protofibrils, and the key differences between the uptake of protofibrils and fibrils are their molecular size and morphology. We noted that native Ure2 was also taken up into cells, and this may be an important route to entry for Ure2 aggregates. We also showed that the uptake of protofibrils and fibrils resulted in cell toxicity, which was most marked in the case of protofibrils. Finally, we showed that clathrin heavy chain is necessary for the uptake of protofibrils and fibrils. Representative photomicrographs from immunofluorescence assays. The three different states of Ure2: native Ure2 (a), protofibril (b), and mature fibril (c) are seen inside HeLa cells (green) after 0.5 (a, b), 1 (a, c), 4 (b, c), or 10 (a, c) hours of incubation (all data in the figure were recorded after 10 hours of incubation). In some cases, membrane-like structures of Ure2 (a, b, c) were seen inside the cells. Scale bar, 10 m.

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The results showed that the viability of the three concentrations of PrP116 (2.5, 10 and 25µg/ml), protofibrils and native Ure2 (10, 50, 100, 150, 200, 250 and 300µg/ml) did not

significantly change (P>0.05) compared to the control (Fig. 6). However, PrP106 (20, 50 and 100µg/ml), PrP106S16C (50 and 100µg/ml), PrP106S16C/116Q (10, 50, 100, 200 and 300µg/ml) and PrP106S16C/116Q/Ure2 (10, 50, 100, 150, 200, 250 and 300µg/ml) significantly reduced the viability of cells (Fig. 6) (P 5ec8ef588b

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