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We use this protocol and it's working

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Anti-GFP pull-down with syringe filter partition

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ABSTRACT

Aggregation of proteins containing expanded polyglutamine (polyQ) repeats is the cytopathologic hallmark of a group of dominantly inherited neurodegenerative diseases, including Huntington's disease (HD). Huntingtin (Htt), the disease protein of HD, forms amyloid-like fibrils by liquid-to-solid phase transition. Macroautophagy has been proposed to clear polyQ aggregates, but the efficiency of aggrephagy is limited. Here, we used cryo-electron tomography to visualize the interactions of autophagosomes with polyQ aggregates in cultured cells *in situ*. We found that an amorphous aggregate phase exists next to the radially organized polyQ fibrils. Autophagosomes preferentially engulfed this amorphous material, mediated by interactions between the autophagy receptor p62/SQSTM1 and the non-fibrillar aggregate surface. In contrast, amyloid fibrils excluded p62 and evaded clearance, resulting in trapping of autophagic structures. These results suggest that the limited efficiency of autophagy in clearing polyQ aggregates is due to the inability of autophagosomes to interact productively with the non-deformable, fibrillar disease aggregates.

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Sample lysis and filter partition

1 Htt64Q-GFP induced with Muristerone A for polyQ expression in Neuro2a, for two days in a 15cm dish

2 wash with PBS, and lysed in cold isotonic buffer (0.25M sucrose, 1mM EDTA, 20mM HEPES pH 7.4) with protease inhibitor, with a gauge 22-needle for 10 times.

3 Nuclei were pelleted at 800g for 10min at 4°C

4 supernatant was then centrifuged at 16000g for 10min at 4°C to pellet aggregates and the associated proteins

5 The pellet was washed once in PBS to remove soluble Htt64Q-GFP and was then dissolved in PBS, and passed through pre-equilibrated syringe filters (in the same buffer) with 0.2um or 0.4um pore sizes (Millipore).

6 The filter blocked fractions were then collected by washing it with a syringe, in the same buffer, and pelleted again at 16,000g for 10min at 4°C.

Anti-GFP pull down

7 The fractions were then incubated with magnetic GFP-Trap (ChromTek) for 6 h at 4°C in binding buffer (140 mM NaCl, 25 mM Tris pH 7.6–8.0, 0.5% NP40, 1 mM EDTA) and 2% BSA (Sigma).

8 Lysates from cells without muristerone A induction was used as negative control in pull down.

9 After 5 washes with the binding buffer, the bound fraction was eluted and denaturation in sample buffer for dot blot analysis.