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Sample vitrification for cryo-ET

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We use this protocol and it's working

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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.

Troubleshooting

vitrification for cells grown on the grids

1 Cells were seeded on holey carbon-coated 200 mesh gold EM grids (Quantifoil Micro Tools, Jena, Germany) in 35 mm cell culture dishes.

2 Allow to properly attach before transfection with GFP-polyQ and RFP-reporters. Cells were incubated for 1 day before media change, and followed by another day of incubation before drug treatments.

3 Prior to vitrification, cells were applied with DMEM containing 10 % glycerol as a **cryo-protectant** and Dynabeads (Invitrogen) at 1:40 dilution for 3D CLEM workflow. Dynabeads maybe skipped of the fluorescent target is large, such as the polyQ-GFP aggregates which span several microns and are visible in SEM and FIB views for targeted milling.

4 The grids were then mounted on Vitrobot Mark IV (Thermo), blotted from the back side using FEI Vitrobot PerforatedFilter Paper (Whatman) with force 10 for 15 seconds (this setting is specific to our vitrobot and may vary from place to place) at room temperature, and plunged into a 2:1 ethane:propane mixture cooled down to liquid nitrogen temperature.

5 Plunge-frozen grids were then clipped into Autogrid support frames modified with a cut-out (Thermo), stored in liquid nitrogen, and maintained at ≤−170°C for all steps.

vitrification for isolated vesicles containing polyQ-GFP and RFP-LC3B

6 The vesicles (~200ul collected from 1 of 6-well containing cells) were re-suspended in 1X PBS and passed through membrane filter (Corning) to remove large clumps prior to vitrification.

7 4 ml of the sample was applied to glow discharged holey carbon-coated 200 mesh copper EM grids (Quantifoil Micro Tools), vitrified as above at 4 °C and 100 % humidity.