Total proteome turbidity assay for tracking global protein aggregation in the natural cellular environment

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Supplementary information

Measuring protein aggregation by turbidity is a well-known method. The main purpose, and novelty, of the modifications we describe for this method, is to allow assessment of the propensity for aggregation of proteins without the need of purifying them from the sample. Usually, turbidity measurements require large volumes of at least 10 μM final protein concentration [1]. However, the yields of many protein preparations are too low to allow screening which requires diluting it in buffer. In our protocol soluble proteins are extracted from cells using well-established extraction procedures.

The protocols we employed for extraction are the standard commonly used protocols specific for each tissue/organism used.

We remove, by centrifugation, aggregates as well as membrane remnants and all other non-soluble components from the tissue, and we continue to work with the remaining clear supernatant.

Certain soluble molecules such as lipids may be present in the soluble fraction of the extract. However, they will be present also in the control samples *i.e.* treated *vs.* untreated tissues/organisms, or as in the present paper in brains of AD *vs.* healthy persons; in flies expressing aggregative protein (tau or Aβ, VHL) *vs.* same flies NOT expressing them; and in cell culture expressing *vs.* non expressing TDP-43. We measured the turbidity of total soluble protein fraction from Drosophila (Wild type flies) and in parallel we employed proteinase K and trypsin, commonly used proteases, for monitoring aggregation dynamics. The following **Figure S2** shows that upon treating the soluble protein fraction with either proteinase K or trypsin before turbidity measurements - no turbidity is observed. Note, trypsin is more sensitive to protease inhibitors which are present in the total soluble protein fraction. To overcome this problem we washed the protein sample on vivacon 500 with 10 kDa cut-off and saved the flow through. Proteins were trypsinized and the peptides were eluted from the column in low volume. The flow through was added back to the peptides and turbidity was measured (Figure S2 A). In a second experiment we extracted proteins from 20 flies with and without protease inhibitors, working at high protein concentration aimed at enhancing turbidity levels. After 19 hours proteinase K or trypsin were added to the aggregative sample. After adding proteases we could detect reduction in turbidity levels due to aggregate proteolysis. Whereas proteinase K reduced the signal to the starting level, trypsin treatment caused a reduction of the turbidity only by half (**Figure S2 B**). We suspect that the differences between the proteases are due to protease concentration and buffer conditions.

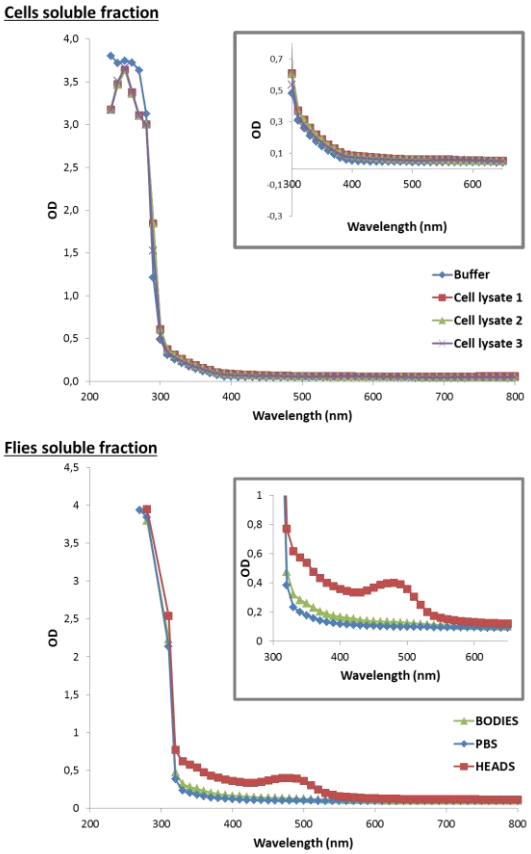

Figure S1. Spectra of global proteome absorbance of soluble fraction from cells or flies. Absorbance spectra of total soluble protein extracts (1 mg/ml) from cells or flies 250–800 nm.

Figure S1 reveals no significant peak at 340 or 600 nm. Note that the absorbance intensity is higher using fly heads than total body due to the pigment present in the eyes (but this occurs also in the control). The peak is around 450–550 nm as expected from a red color.

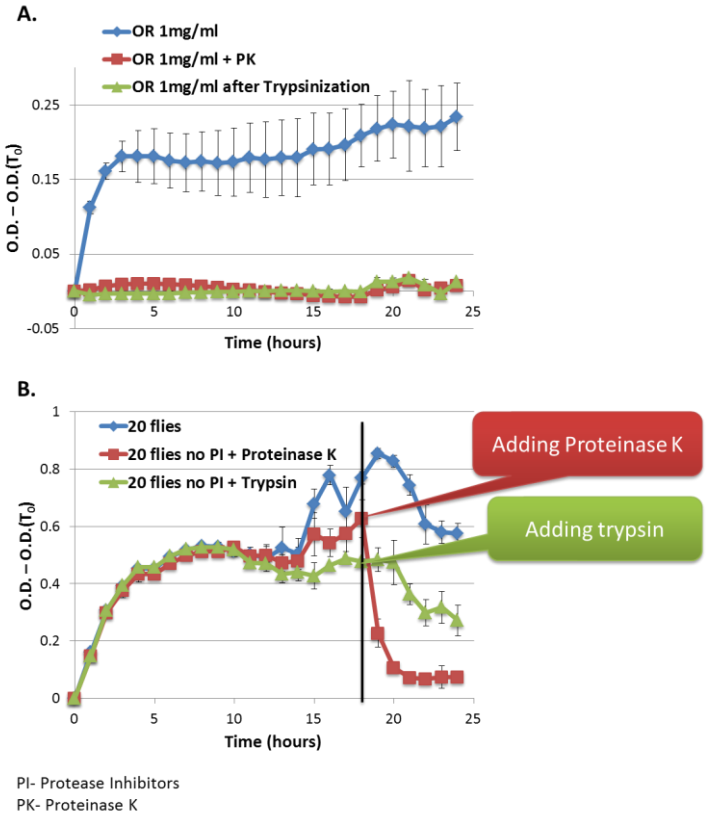

Figure S2. Global proteome turbidity of wild type flies. (**A**) Turbidity levels of soluble protein extracts (1 mg/ml) from flies in the presence of proteinase K or following trypsin treatment. (**B**) Turbidity levels of soluble protein extracts from 20 flies with and without protease inhibitors. The black line represents the time point of adding proteinase K or trypsin to the samples that do not contain protease inhibitors. All optical densities were measured at 340 nm and their first measurement (O.D.(T0)) was subtracted in order to eliminate background absorbance of the samples.

The following experiment illustrates this point:
We examined, following centrifugation, the clear supernatant and the pellet fractions from three independently prepared human brain tissue extracts.

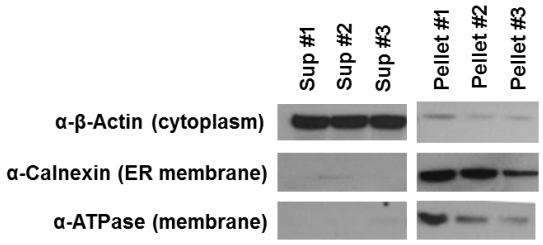

Figure S3. Western blot of the clear supernatant and the pellet fractions from three independently prepared human brain tissue extracts.

Samples were reacted with an antibody against actin (which is present in the cytoplasm), an antibody against calnexin (which is present on the ER membrane) and an antibody against ATPase (which is present on cell membranes). As seen if **Fig S3** the supernatant fraction contains soluble proteins but not membrane or ER proteins (insoluble).

References

1. Sarciaux JM, Mansour S, Hageman MJ, Nail SL. Effects of buffer composition and processing conditions on aggregation of bovine IgG during freeze-drying. J Pharm Sci. 1999;88(12):1354-61. PubMed PMID: 10585234.