Supplemental Materials

Molecular Biology of the Cell

Cenini et al.

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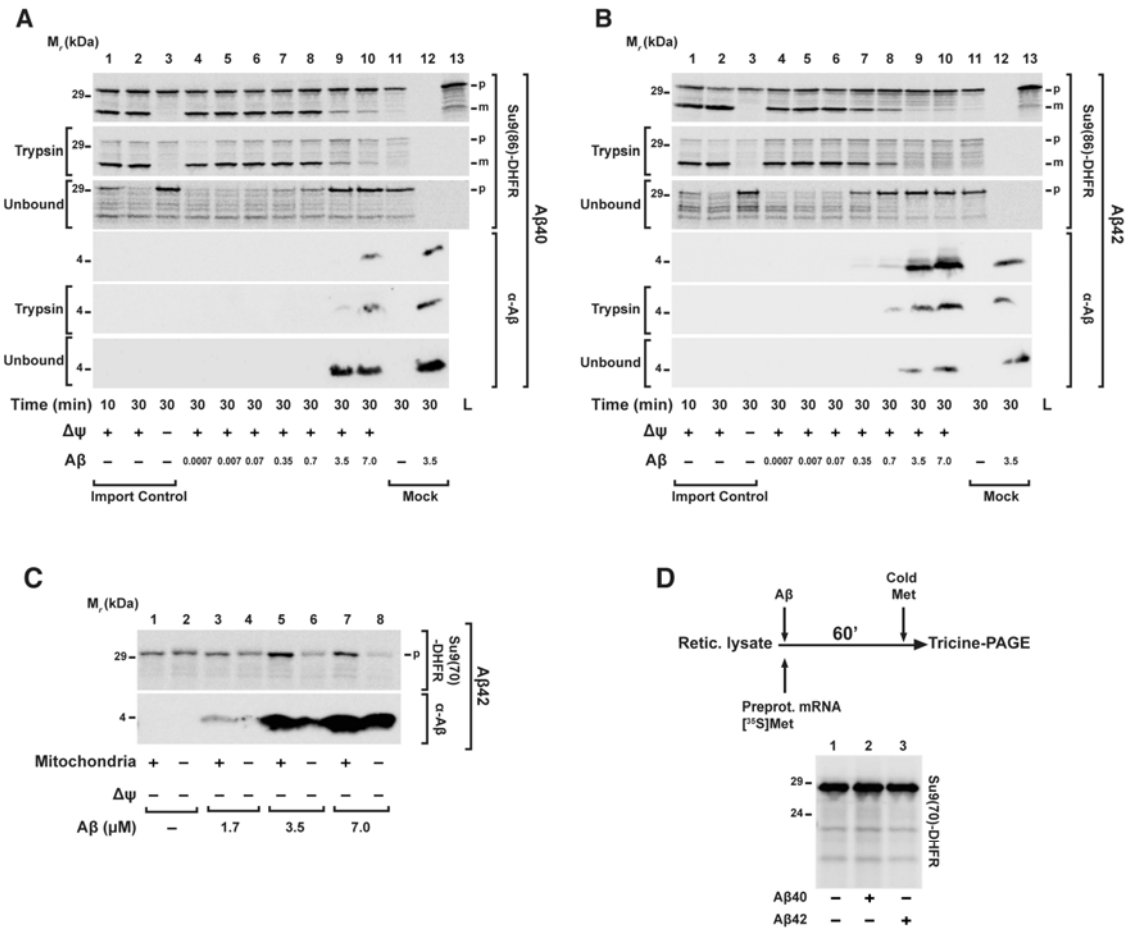
"Amyloid β-peptides interfere with mitochondrial preprotein import competence by a co-aggregation process"

Supplementary figure legends

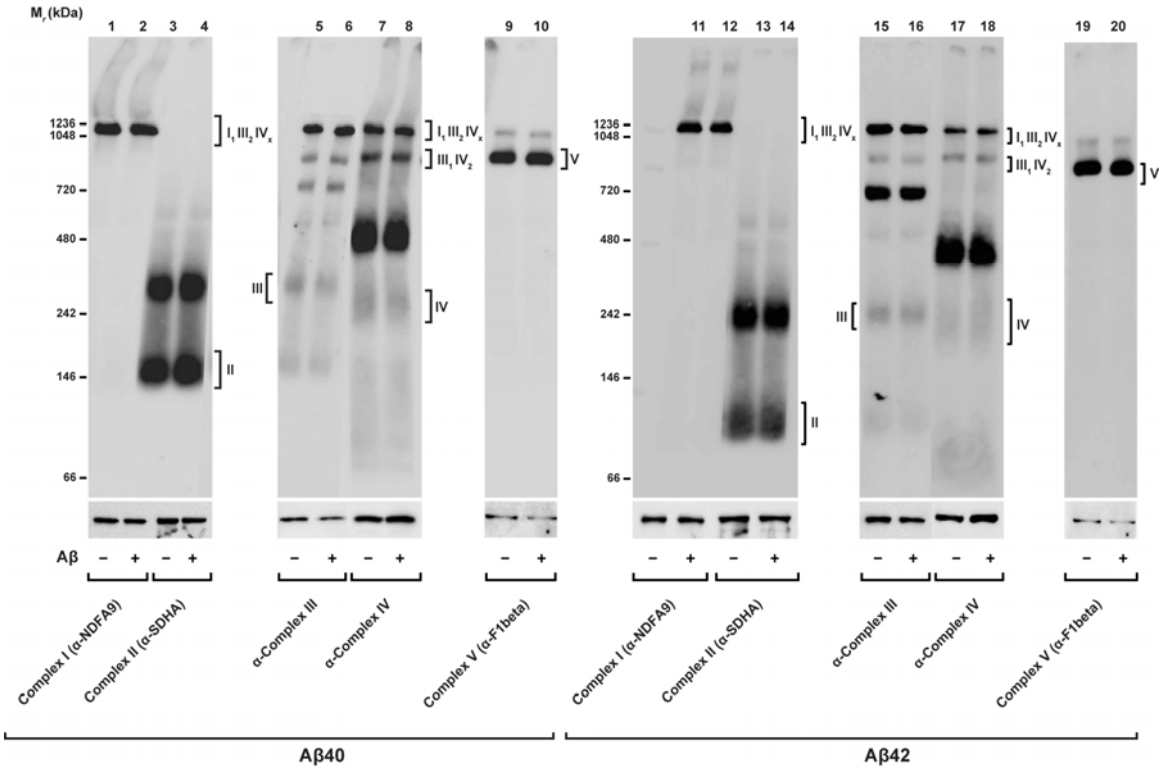
Figure S1. **Concentration dependency of Aβ peptide-inhibition of mitochondrial import**. (**A**, **B**) Energized and intact mitochondria were incubated with mitochondrial precursor protein [^{35}S]Su9(86)DHFR and the indicated amounts of Aβ40 (**A**) and Aβ42 (**B**) for 30 min at 30 °C. Half of the samples were incubated with trypsin (*100 µg/ml*) to digest the precursor proteins not imported. Mitochondrial pellets and respective supernatants were loaded on tricine-SDS-PAGE followed by Western blot, digital autoradiography and immunodecoration against Aβ peptides. *p*, precursor form; *m*, mature form; *L*, loading control; *Mock*, control reaction in absence of mitochondria. **(C)** Isolated mitochondria without $\Delta\psi_{mit}$ were incubated with increasing amounts of Aβ40 and Aβ42 (as indicated) and precursor protein [^{35}S]Su9(70)DHFR. **(D)** Aβ peptide effect on translation. Rabbit reticulocyte lysate, Su9(70)DHFR mRNA, [^{35}S]methionine were incubated with or without Aβ peptides (3.5 µM) at 30° C for 60 min. The translation was stop adding 8 mM cold methionine. The samples were analyzed by tricine-SDS-PAGE followed by Western blot and digital autoradiography.

Figure S2. **Effect of Aβ peptides on mitochondrial respiratory chain complexes structure and composition.** Intact and energized mitochondria were treated with Aβ peptides (3.5 µM). Structure and composition of the mitochondrial respiratory chain complexes were analyzed by SDS-PAGE, BN-PAGE and Western blot. Before loading the gels, mitochondria were solubilized in a buffer containing 1% digitonin. Immunodecorations against components of respiratory complexes I, II, III, IV, and V were performed (see "Material and Methods"). Indicated are the bands representing the respective respiratory complexes or super-complexes.

Figure S3. **Co-aggregation between Aβ peptides and the genuine mitochondrial precursor protein ornithine carbamoyltransferase (OTC)**. Precursor protein [^{35}S]OTC was incubated in import buffer in presence or absence of Aβ peptides in the indicated amounts. After incubation, samples were analyzed by a filter retardation assay. Samples were filtered directly through cellulose acetate membrane and nitrocellulose membrane using a dot blot filtration unit as described in "Material and Methods" section. Proteins on both membranes were colored with Ponceau S followed by digital autoradiography to detect the precursor protein [^{35}S]OTC signal and by immunodecoration to detect the Aβ peptides.



Cenini *et al.*, Supplm. Fig. S1



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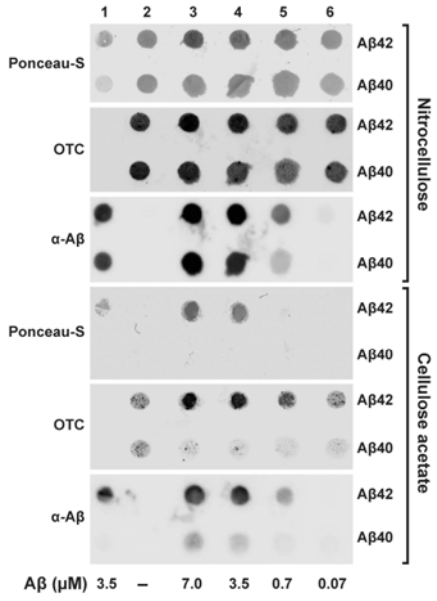

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