Electronic Supplementary Information (ESI)

Real-Time Investigation of Cytochrome c Release Profiles in Living Neuronal Cells Undergoing Amyloid Beta Oligomer-Induced Apoptosis†

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Contents:

Materials and Methods

1. Amyloid beta oligomer (AβO) preparation

Soluble oligomers of amyloid beta 1-42 ($A\beta_{1\text{-}42}$) were prepared according to a previous protocol.[1] In brief, $A\beta_{1\text{-}42}$ (Anaspec) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma) at 1 mM, sonicated for 5 minutes, and aliquoted into the tubes. The samples were dried with a gentle nitrogen stream and placed in a vacuum chamber to completely remove organic solvent, and stored under -20ºC. For AβO preparation, the dried $A\beta_{1\text{-}42}$ was dissolved in dimethyl sulfoxide (DMSO) (Sigma) at 5 mM, and phenol red-free DMEM/F12 medium (Invitrogen) was added. The sample was vortexed for 1 minute, and then incubated at 4ºC for 24 hours.

2. PRET probe preparation

Mercaptopropionic acid-modified gold nanoparticles (MPA-GNPs) were prepared for detection of intracellular Cyt c.[2] One mL of GNPs (50 nm, Ted Pella Inc.) was mixed with 10 µL Tween-20 (Sigma). The mixture was vortexed thoroughly and incubated at 4ºC for 1 hour. Ten µL MPA was added to the mixture and vortexed. The solution was incubated at 4ºC for 5 hours, and then the MPA-GNPs were washed with Milli-Q water (Resistance =18 mΩ) two times. The particles were collected by centrifugation (5,000 rpm, 30 minutes) for each washing step.

3. Cell culture

Human neuroblastoma SH-SY5Y cells were incubated in the CO_2-independent medium (Invitrogen) supplemented with 10% FBS (Invitrogen), 1% Non-essential amino acid solution (Gibco) and 1% penicillin-streptomycin (Sigma). For the PRET experiments, $4x10^5$ cells were seeded onto tissue culture petridish (60 mm in diameter, BD) with 5 mL culture medium. Twenty four hours after seeding, 10^5 MPA-modified gold nanoparticle probes were added into the medium, followed by 12 hours of incubation to allow cellular uptake of added MPA-GNPs. Afterwards, cells were washed twice with fresh medium to remove the extraneous particles and then incubated for an additional 12 hours. AβO was added to the culture medium at the designed concentration. For control experiments, vehicle (trace amount of DMSO) was used as controls. For the nerve growth factor (NGF)-experiment, cells were incubated with 50 ng/mL of NGF (Progmega) for 24 hours prior to the introduction of AβO. Final DMSO concentration in cell culture medium was 0.05 v/v% for test and control experiments, which is not substantially toxic to SH-SY5Y.

4. Apoptosis analysis using Annexin-V FITC staining

To evaluate apoptosis, Annexin-V fluorescein isothiocyanate (FITC) (Sigma) was used to stain apoptotic cells. According to the manufacturer's protocol, Annexin-V FITC was diluted into the staining buffer (5 μL in 1 mL each). The solution (100 μL) was added into each well and incubated for 10 minutes. The cells were carefully washed twice with phosphate-buffered saline (PBS, Invitrogen) and fixed with 4% para-formaldehyde solution (20 minutes). Phase contrast and fluorescence images were acquired using a fluorescence microscope (TE 2000-E, Nikon Instruments).

5. Dark field microscopy and PRET

The microscopy system consisted of an inverted microscope (Carl Zeiss Axiovert 200) equipped with a dark-field condenser (1.2-1.4 numerical aperture) and a white light source (Xenon Arc Lamp, Storz 300 W). Images were acquired using a digital camera (Q-color3, Olympus) Scattering spectra from the probes at different positions were collected using a monochromator (300 mm focal length and 300 grooves per mm, Acton Research) with a cooled spectrograph CCD camera (Roper Scientific). A 2 μm-wide aperture was placed in front of the monochromator to keep only a single probe in the region of interest. For the PRET measurement from living cells, the cell culture plate was mounted onto the microscope and the spectra from the intracellular probes were obtained with the same method.

6. Spectral analysis

The base line subtraction and normalization of the achieved scattering spectrum were accomplished with the equation, $I=(I_{probe\ in\ cell} - I_{baseline}) / (I_{probe\ in\ medium} - I_{baseline})$, where $I_{probe\ in\ cell}$ and $I_{probe\ in\ medium}$ are an intensity obtained from the probe inside a cell and outside a cell (in medium), respectively. $I_{baseline}$ was obtained in the absence of any light sources. The spectrum was then processed by the adjacent averaged method using Origin software. For semi-quantification for Cyt c release, changes in quenching dip intensities at 520-530 nm were calculated by the subtraction with its initial intensity, $\Delta I = |(I_{t=0} - I_t) / I_{t=0}|$.

References

1. R. Bravo, M. Arimon, J. J. Valle-Delgado, R. Garcia, N. Durany, S. Castel, M. Cruz, S. Ventura, X. Fernandez-Busquets, *J. Biol. Chem.* 2008, **283**, 32471.
2. Y. Choi, T. Kang, L. P. Lee, *Nano Lett.* 2009, **9**, 85.

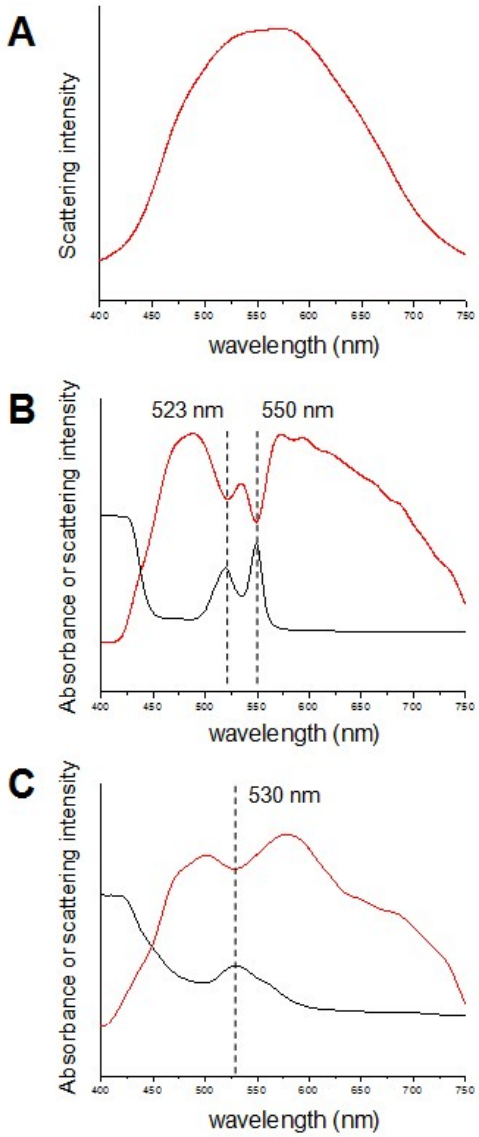

Fig. S1 Plasmon resonance energy transfer (PRET) technique for Cyt c detection. (A) Optical spectrum of a 50 nm sized gold nanoparticle has intensive scattering from 450 to 700 nm. (B) Scattering spectrum (red) and absorbance spectrum (black) of reduced Cyt c (100 μM in PBS (pH 7.2)), showing strong quenching dips and absorption peaks at 523 and 550 nm. (C) Scattering spectrum (red) and absorbance spectrum (black) of oxidized Cyt c (100 μM in PBS (pH 7.2)), which resulted in a quenching dip and absorption peak at the same wavelength (530 nm) Thus, characteristic dips in scattering spectra can be used to analyze the Cyt c.

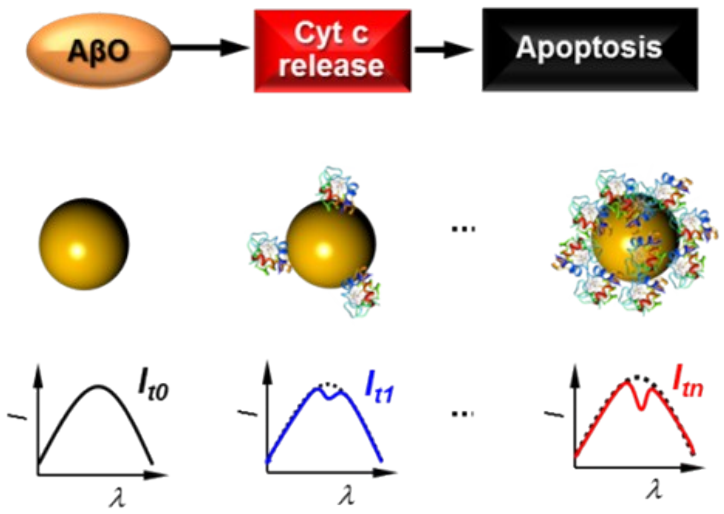

Fig. S2 Schematic of monitoring and analysis of AβO-induced cytosolic Cyt c release using a PRET-based spectroscopy. AβO-induced apoptosis leads to increase in Cyt c level in the cytosol, which causes a quenching dip in the PRET spectrum.

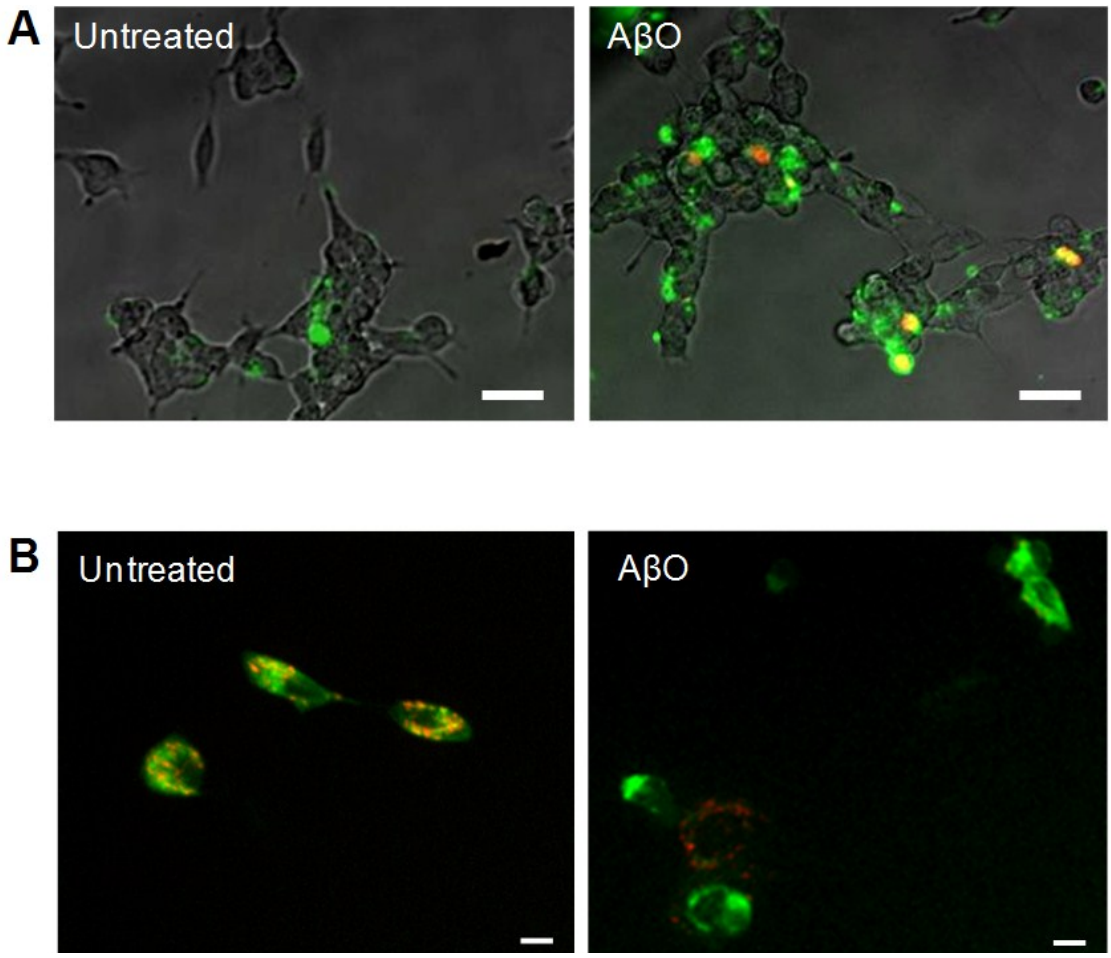

Fig. S3 Immunofluorescence for studies on the apoptotic effects of A*β*O on SH-SY5Y cells. (A) Immunostaining with FITC-conjugated Annexin V (green) and propidium iodide (red). Cells were stained after AβO treatment (0 and 2.5 μm). Annexin V positive and PI positive staining indicates early and late apoptotic stages, respectively. Scale bars = 50 μm. (B) Fluorescence images of JC-1 staining of untreated control cells and the A*β*O-treated cells (3 hours after introduction). JC-1 aggregates on polarized mitochondrial membrane (red) of untreated cells. In contrast, A*β*O leads to the loss of mitochondrial membrane potential. Scale bars = 10 μm.