Supplementary Information
For

Effect of Magnetic Modulation of Mitochondrial Voltage-Dependent Anion Channel 2 against beta-Amyloid induced Neurotoxicity

Sujin Lee[a], Junghoon Lee[a], Sang Jun Sim[b], Misun Cha[b*]

[a] *School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, 151-744, South Korea.*
[b] *Department of Chemical Engineering and Biological Engineering, Korea University, Seoul, 136-701, South Korea.*

* CORRESPONDING AUTHOR FOOTNOTE

Department of Chemical and Biological Engineering, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, Repulic of Korea.

Fax:+82-2-3290-3686; Tel: +82-2-3290-3686; E-mail: cmsbestok@gmail.com

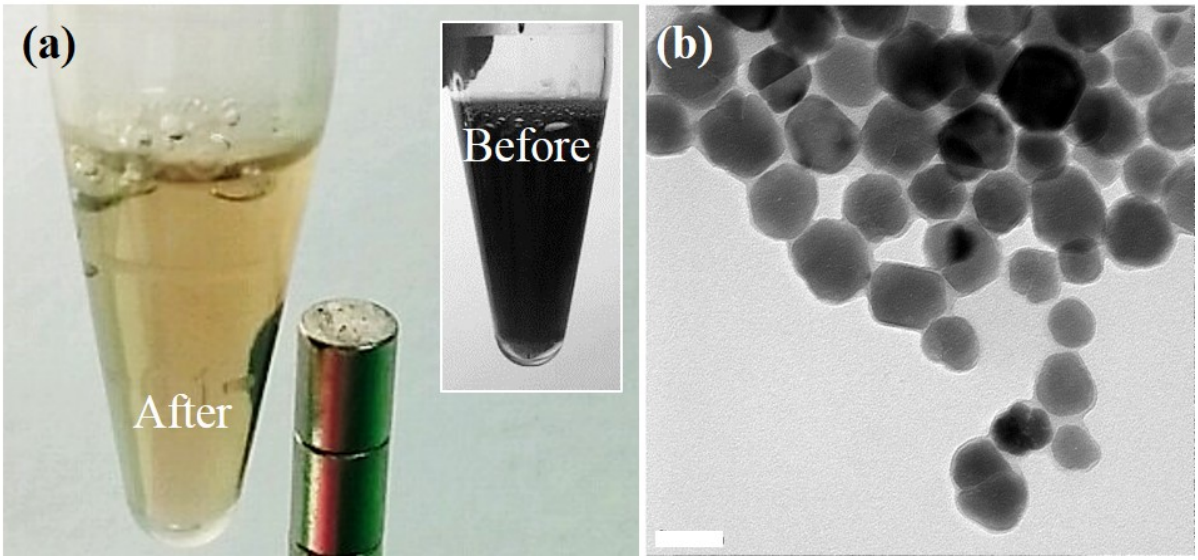

Fig. S1 (a) Magnetic attraction of BMP disperse in 1X PBS, (b) Transmission electron microscopy (TEM) image of BMP with a scale bar 50 nm. The BMPs from the *Magnetospirillum* sp. AMB-1 were used in our approach, which are inherently biocompatible, can be effectively conjugated with other biomolecules, and can be dispersed well in aqueous solutions because of their stable lipid membrane surrounding the magnetic core.

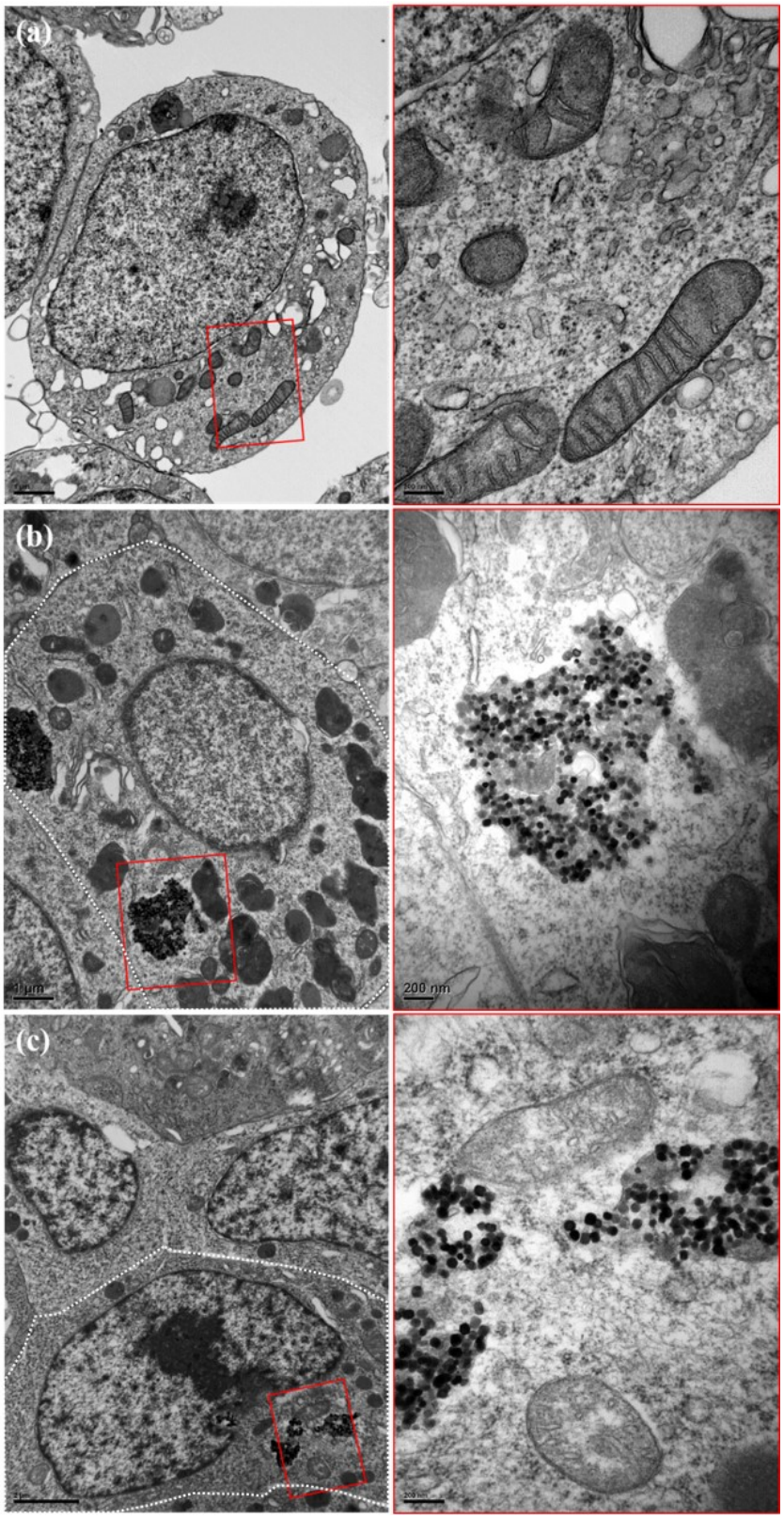

Fig. S2 Transmission electron microscopy (TEM) image of BMPs internalized by a SH-SY5y cells. (a) Control, (b) BMPs only, and (c) BMPs-antibody conjugate.

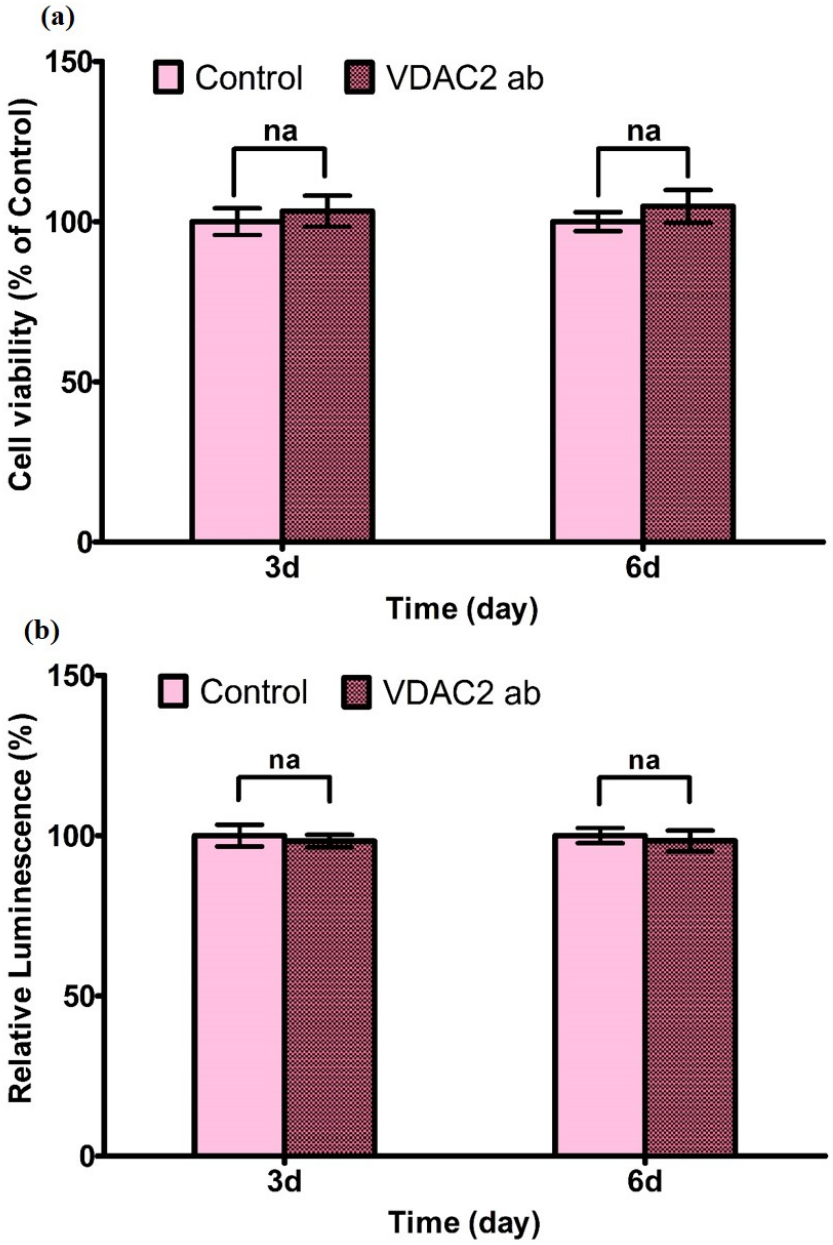

Fig. S3 Effect of 10 μg/ml VDAC2 antibody treatment on cell growth by (a) MTS assay and (b) ATP level. (na: no significant difference, n=3)

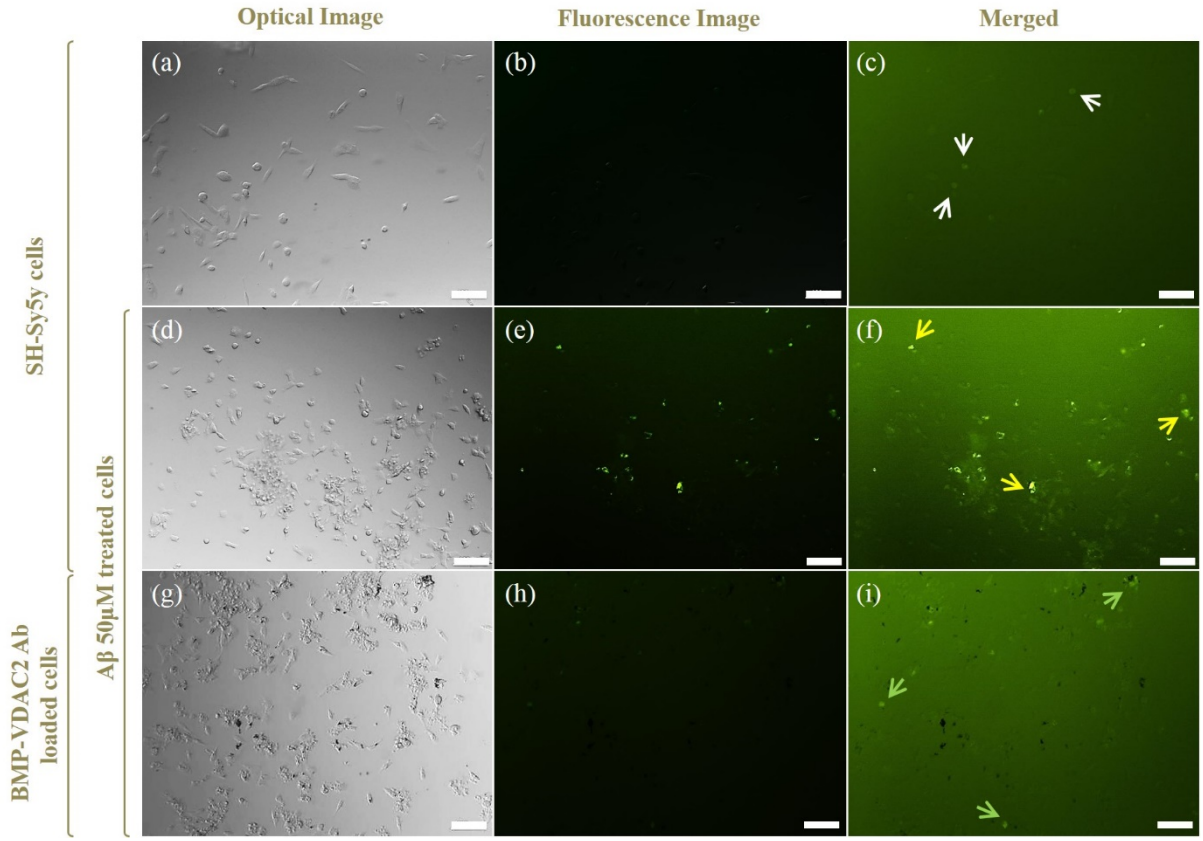

Fig. S4 (a) Optical, (b) fluorescence and (c) optical – fluorescence combine images of normal SH-Sy5y cells; (d) optical, (e) fluorescence and (f) optical – fluorescence combine images of β-amyloid treated normal SH-Sy5y cells; and (g) optical, (h) fluorescence and (i) optical – fluorescence combine images β-amyloid treated BMP-Ab loaded SH-Sy5y cells. It is clear that Ca^{2+} level of β-amyloid treated normal cells is much higher than level of BMP-Ab loaded cells ((f), (i)).

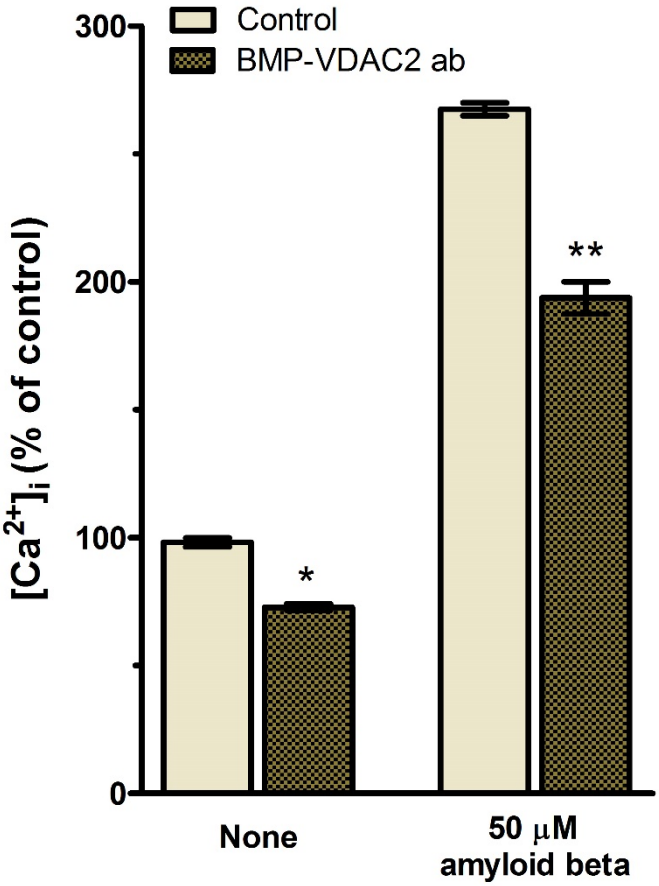

Fig. 5S Effect of BMP-VDAC2 ab complex to reduce calcium influx induced by amyloid beta. Calcium level was calculated from the calibrated fluorescence signal of the probe fluo-3. *$p<0.05$ and **$p<0.01$ compared with the control cell.

Materials and methods

1. Preparation of BMPs

BMPs were obtained from *Magnetospirillum* sp. AMB-1 (ATCC® 700264), which was cultured in magnetic spirillum growth medium (MSGM) for 5 days inside shaking incubator at 30 ℃ under anaerobic conditions. Cultured *Magnetospirillum* sp. AMB-1 were centrifuged for 25 min at 5000 rpm and then lysed by sonication (VCX500, Sonics&Materials, USA) for 30 min. BMPs were collected using a neodymiumiron boron (NdFeB) magnet and washed for 5 times with 1X PBS. Collected BMPs were dispersed in 1X PBS and sterilized with autoclave (121 ℃, 15 min). Concentration of extracted BMPs was determined by inductive coupled plasma-atomic emission spectrometer (ICP-AEX, ICPS-7500, Shimadzu, Japan).

2. Conjugation of VDAC2 antibody to BMPs

N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride(EDC, Sigma-aldrich, Korea) and N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS, Sigma-aldrich, Korea) were dissolved in 0.1 M MES buffer (2-(N-morpholino) ethanesulfonic acid, pH=8.3). The mixture was added to 10 μl of 1 mg/ml C-termini VDAC2 antibody to be able to react with a carboxyl group for 15 min to form an amine-reactive O-acylisourea intermediate. After adjustment of pH in between 7 to 8 by adding 3 μl of 20X PBS buffer, 500 μl of BMPs contained in 1X PBS is added. This mixture is gently shaken at room temperature for 2 h to accelerate binding reaction. Unconjugated antibodies were separated from the antibody conjugated BMPs three times with 1X PBS buffer under the strong magnet field. Bicinchoninic acid (BCA) protein assay was demonstrated to determine the amount of antibody bound to nanoparticle (ref 1). BCA protein assay result showed that the amount of antibody bound to BMP is around 60%.

Ref 1: S.K.Vashist, Comparison of 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Based Strategies to crosslink Antibodies on Amine-Functionalized Platforms for Immunodiagnostic Applications, *Diagnostics*, 2012, **2**, 23-33.

3. Preparation of aged $A\beta_{25-35}$

$A\beta_{25-35}$ was dissolved in distilled water at a concentration of 1 mM and then incubated in a capped

vial at 37 ℃ for 7 days to form aggregation, which was frozen and stored at 4 ℃.

4. Cell culture and cellular uptake of BMPs

Human neuroblastoma SH-SY5Y cells were cultured in Low glucose Dulbecco's modified Eagle's medium (DMEM/LOW GLUCOSE, Thermo Scientific, USA) at 37 ℃ with 5 % of CO_2 to reach a confluence of 70 % to 80 % containing 10 % (v/v) fatal bovine serum (FBS, Thermo Scientific, USA) and 1 % penicillin/streptomycin (Thermo Scientific, USA). The BMPs and VDAC2 antibody conjugated BMPs were treated in the serum free medium with 10 μg/ml concentration during 6 h for sufficient time to internalize. And then they were cultured in normal cell culture medium for 24 h to take enough time to deliver particles into the intracellular organelles. After trypsinization, magnetic particle loaded cells were separated from non-loaded cells and collected using strong neodymium magnet. Collected magnetic particle loaded cells were seeded at 1 x 10^4 cells/well in 96-well plate after cell enumeration using a hemocytometer.

5. $A\beta_{25-35}$ treatment and measurement of the intracellular calcium level

To induce cell injury, cells were incubated with 50 μM $A\beta_{25-35}$ for 24 h. To study the effects of BMP-VDAC2 antibody complex, cells were pre-incubated with particles for 3 days, and then $A\beta_{25-35}$ was added to the medium of magnetically collected cells for additional 24 h.

To measure the intracellular calcium level of BMP-VDAC2 antibody loaded and normal SH-SY5Y cells, cells grown on glass coverslip were treated for 30 min at 37 °C with 5 μM Fluo-3 AM. The fluorescence changes determined by Fluo-3 represent the intracellular calcium $[Ca^{2+}]_i$ changes. Multiple Plate Reader (Victor 3, Perkin Elmer, USA) were acquired fluorescence level using a 488-nm laser source to excite Fluo-3. The signals were collected at 505-530 nm. We calibrated fluo-3 signals to $[Ca^{2+}]_i$ for untargeted SH-SY5Y cell. Using the equation: $[Ca^{2+}] = K_d (F-F_{min})/(F_{max}-F)$, the resting calcium level in SH-SY5Y was 138±3 nM when the K_d of fluo-3 at 37 ℃ was found to be 864 nM. (ref 2)

Ref 2: Merritt JE et al, Use of fluo-3 to measure cytosolic Ca^{2+} in platelets and neutrophils, *Biochem J.*, 1990; **269(2)**, 513–519

6. Fluorescence labeling

In order to check accurate targeting of BMPs-VDAC2 antibody complex onto intercellular organelle, VDAC2 antibody was labeled with the fluorescein isothiocyanate (FluoroTag™, Sigmaaldrich, Korea) which is a widely used fluorophore prior to the immobilization of antibody and BMPs. After delivering of FITC labeled BMPs-antibody complex into the SH-SY5Y cells for 24 h, cells were fixed with 3.7 % formaldehyde in 1X PBS for 15 min at room temperature. Following washing twice with wash buffer, 0.1 % Triton X-100 in 1X PBS, the fixed cells were permeabilized and blocked with 1 % BSA and 0.1 Triton X-100 in 1X PBS for 20 min. Then the actin filaments were stained by incubating cells with TRITC-conjugated phalloidin (1:200) for 50 min at room temperature. The cells were washed three times with 1X PBS and nucleus staining was performed by incubating three times with a diluted DAPI in 1X PBS (1:1000) for 5 min each.

7. Confocal Microscopy

Other staining procedures were same as above described cell staining. The stained cells were examined using a confocal laser scanning microscope (Carl Zeiss-LSM510, Germany).

8. MTS assay

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was used to evaluate the cytotoxicity of the particles and viability of cells in several conditions. 1 x 10^4 cells/well of SH-SY5Y cells were seeded on 96-well plate with 100 μl of the culture medium. After incubation for 6 days on each condition, 20 μl of CellTiter 96® Aqueous One Solution Reagent (Promega, USA) was added into each well. Then the plate was incubated at 37 ℃ for 2 h in a humidified 5 % CO_2 atmosphere and absorbance was recorded at 490 nm with an absorbance reader (Thermo Fisher Scientific Inc., USA). All of the assays were repeated at least twice and 3 wells for one group were investigated in each assay. The absorbance of control cells was normalized to 100 % of viability in every assay.

9. ATP assay

The SH-SY5Y cells (1 x 10^4 cells/well) were seeded on opaque-walled 96-well plate with 100 μl of

the culture medium and cultured for 3 and 6 days. ATP content was measured in accordance with the protocol of CellTiter-Glo Luminescent cell viability assay kit (Promega, USA). The procedure was followed a protocol provided by manufacturer. In brief, 100 μl of CellTiter-Glo reagent was added to the each well and mixed for 2 min at room temperature. Then the intracellular ATP content was measured using luminometer (Thermo Fisher Scientific Inc., USA). The assay was repeated twice and 3 wells for one group were investigated in each experiment.