# Supplementary Information

## 'Dunking Doughnuts into Cells – Selective Cellular Translocation and *In Vivo* Analysis of Polymeric Micro-Doughnuts'

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### *Formation of Micro-Doughnuts by Dispersion Polymerization*

Polyvinylpyrrolidone ($M_w$ 40,000, 50 mg, 1.25 μmol) was dissolved in 1, 4-dioxane:ethanol (5:95, 5 mL) and stirred under nitrogen. Separately, 2, 2'-azobis(2-methylpropionitrile) (7 mg, 42.6 μmol) was dissolved in styrene (0.5 mL, 4.3 mmol) with 4-vinylbenzylamine hydrochloride (7 mg, 41.3 μmol) in the presence of *p*-divinylbenzene (4.65 μL, 32.6 μmol). The monomer suspension was added to the alcoholic media and the dispersion stirred for 2 hours under nitrogen at 25 ºC. The temperature was increased to 65 ºC and stirred for 18 hours. Micro-doughnuts were obtained by centrifugation (8500 rpm, 3 minutes) and washed sequentially with methanol and water. The particle size was measured on a Beckman Coulter LS 230 in water.

### *Coupling of Aminohexanoic acid to Micro-Doughnuts*

Micro-doughnuts (3% solid content, 1 mL) were washed with dimethylformamide (3 × 1 mL) and resuspended in dimethylformamide (0.5 mL). Separately, Fmoc-aminohexanoic acid (7.4 mg, 21 μmol) was dissolved in dimethylformamide (0.5 mL) in the presence of 1-hydroxybenzotriazole (2.8 mg, 21 μmol) and the solution was stirred for 10 minutes before the addition of diisopropylcarbodiimide (3.3 μL, 21 μmol). The solution was stirred for a further 10 minutes before addition to the micro-doughnuts. The suspension was stirred at 25 ºC for 18 hours. Micro-doughnuts were obtained by centrifugation (13,000 rpm, 30 seconds) and washed sequentially in

dimethylformamide, methanol and water. Fmoc deprotection was achieved in 20% piperidine in dimethylformamide (3 × 1 mL, 20 minutes).

*Labelling of Aminohexanoic Micro-Doughnuts*

Aminohexanoic micro-doughnuts (3% solid content, 1 mL) were washed in dimethylformamide (3 × 1 mL) and resuspended in dimethylformamide (0.5 mL). Separately, the carboxy-fluorophore (12 μmol) was dissolved in dimethylformamide (0.5 mL) with 1-hydroxybenzotriazole (1.6 mg, 12 μmol) and (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (5.8 mg, 11.2 μmol) in the presence of diisopropylethylamine (4.1 μL, 23.5 μmol). The solution was stirred for 1 minute before addition to the micro-doughnuts and stirring at 25 ºC for 18 hours. Micro-doughnuts were obtained by centrifugation (13,000 rpm, 30 seconds) and washed sequentially in dimethylformamide, methanol and water.

*Cell Cultures*

Cells were grown in growth medium as appropriate (10 mL) supplemented with 10% fetal bovine serum, 100 units/mL penicillin/streptomycin and 4 mM $_L$-glutamine in a T75 flask at 37 ºC/5% $CO_2$ until 70% confluency. Cells were harvested with trypsin/EDTA following washing with phosphate buffered saline (PBS, pH 7.4) and collected in the appropriate growth media.
*For uptake experiments*, cells were plated at a density of $4 \times 10^4$ cells per well in a 24-well plate (media volume: 350 μL). After 24 hours, rhodamine B labelled micro-doughnuts (26, 43 and 86 μg/mL) were added as a suspension in sterile water. After 6, 12 and 24 hours flow cytometric analysis was made following cell harvesting in trypsin/EDTA, collection in growth media, pelletization (1000 rpm, 4 min) and finally resuspension in 0.2% trypan blue in Hank's Balanced Salt Solution (HBSS). Analysis was made on a BD FACS Aria™ System using a 488 nm excitation laser and 575/26 band pass filter. Analysis was made using FACS Diva software.
*For competition experiments*, HEK293T and B16F10 cells were plated separately in Dulbecco's Modified Eagle Medium (DMEM, 350 μL) in a 24-well plate and incubated for 24 h at 37 ºC/5% $CO_2$. 500 nm microspheres labelled with dansyl were added to B16F10 cells (86 μg/mL) and Cy5 labelled 500 nm microspheres were added

to HEK293T cells (86 μg/mL). After 24 h, cells were prepared for flow cytometric analysis as described above and the cell pellets collected in DMEM. Cells positive for microspheres were sorted (Cy5 required a 633 nm excitation laser and 660/20 band pass filter; dansyl required a 407 nm excitation laser and 450/40 band pass filter) and collected in DMEM, centrifuged to collect the cell pellet (1000 rpm, 4 min) and resuspended in DMEM. The tagged-cells were mixed and seeded to a 12-well plate. After 24 hours, rhodamine B doughnuts (86 μg/mL) or fluorescein labelled 2 μm microspheres (86 μg/mL) were added to the mixed cell cultures. After a following 24 hours flow cytometric analysis was made as described above to determine which cell contained beads or doughnuts (fluorescein required a 488 nm excitation laser and 530/30 band pass filter).

*For RAW264 microscopy experiments*, cells were plated at a density of $2 \times 10^5$ cells per well on 24 mm glass coverslips inserted inside a 6-well plate (media volume: 1.5 mL). After 24 hours, fluorescein micro-doughnuts (26 μg/mL) were added as a suspension in sterile water. After 1 hour the old media was removed and the cells were washed sequentially with PBS and stained as according to the procedures details below. Cells were imaged on a Zeiss Axiovert 200M pseudo-confocal microscope.

*Staining with Hoescht 33342*

Hoescht 33342 (1 μg/mL) was added in PBS (pH 7.4) to cell cultures previously washed with PBS and incubated at 25 ºC for 10 minutes. Cell cultures were washed sequentially with PBS.

*Staining with Alexa Fluor® 568-Phalloidin*

Cells stained with Hoescht 33342 were fixed in 3% *p*-formaldehyde solution in PBS for 20 minutes. Cultures were then washed with PBS and Alexa Fluor® 568-Phalloidin (1 unit) added in PBS (200 μL) supplemented with 1% Bovine Serum Albumin. Cells were incubated at 25 ºC for 20 minutes before washing with PBS and finally stored in PBS at 4 ºC.