

Figure S1. FCS measurement of A β 42 oligomers. FCS curve recorded in the green channel on a sample consisting of 500 nM HF488 A β 42 + 500 nM HF647 A β 42. The two shortest diffusion times were fixed to $\tau_{D1}=27 \mu s$ and $\tau_{D2}=81 \mu s$ corresponding to free dye and HF488 A β 42 monomers respectively, because the diffusion coefficient of HF488 A β 42 is about three times slower than that of the free dye HiLyte488 (Wennmalm et al, 2015). The fit yielded that 16% of the amplitude belonged to the free dye, 77% to the A β 42 monomer, and 7 % to a species of $\tau_{D3}=470 \mu s$. The slowest component indicates that a few oligomers of A β 42 are present.

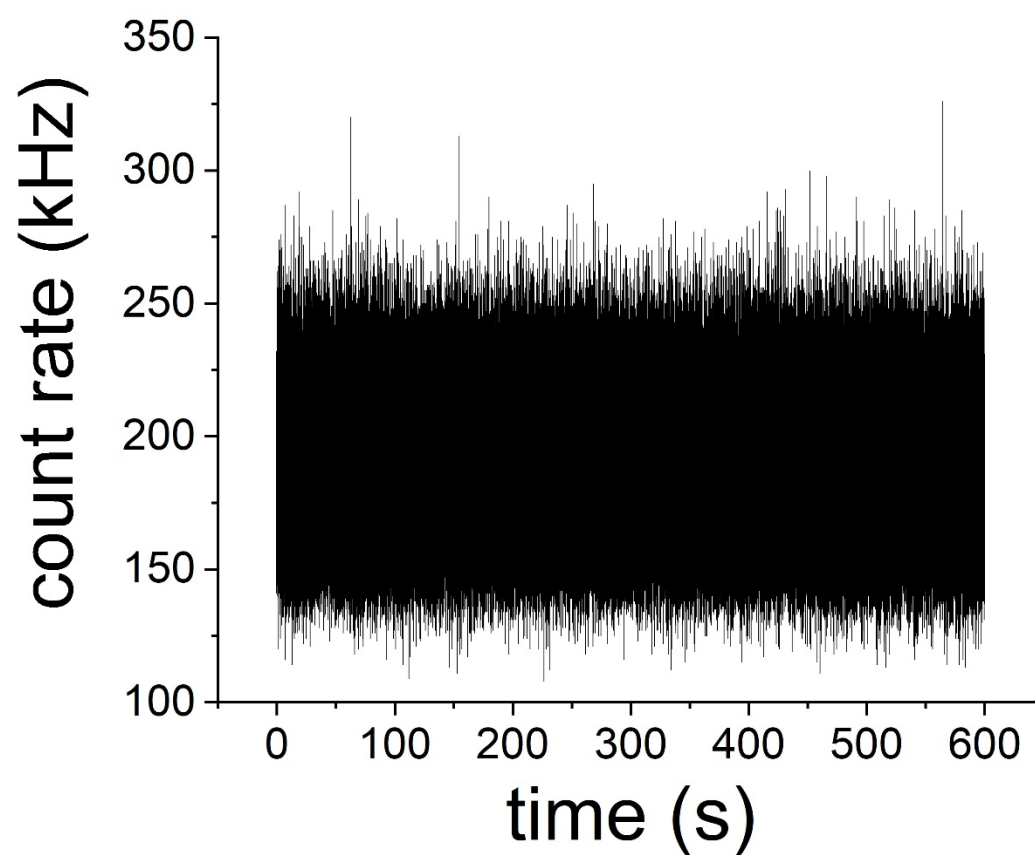


Figure S2. Intensity trace from a 10 min measurement on the same sample as in Figure S1. The lack of spikes in the intensity trace indicates that no large aggregates of A β 42 are present.

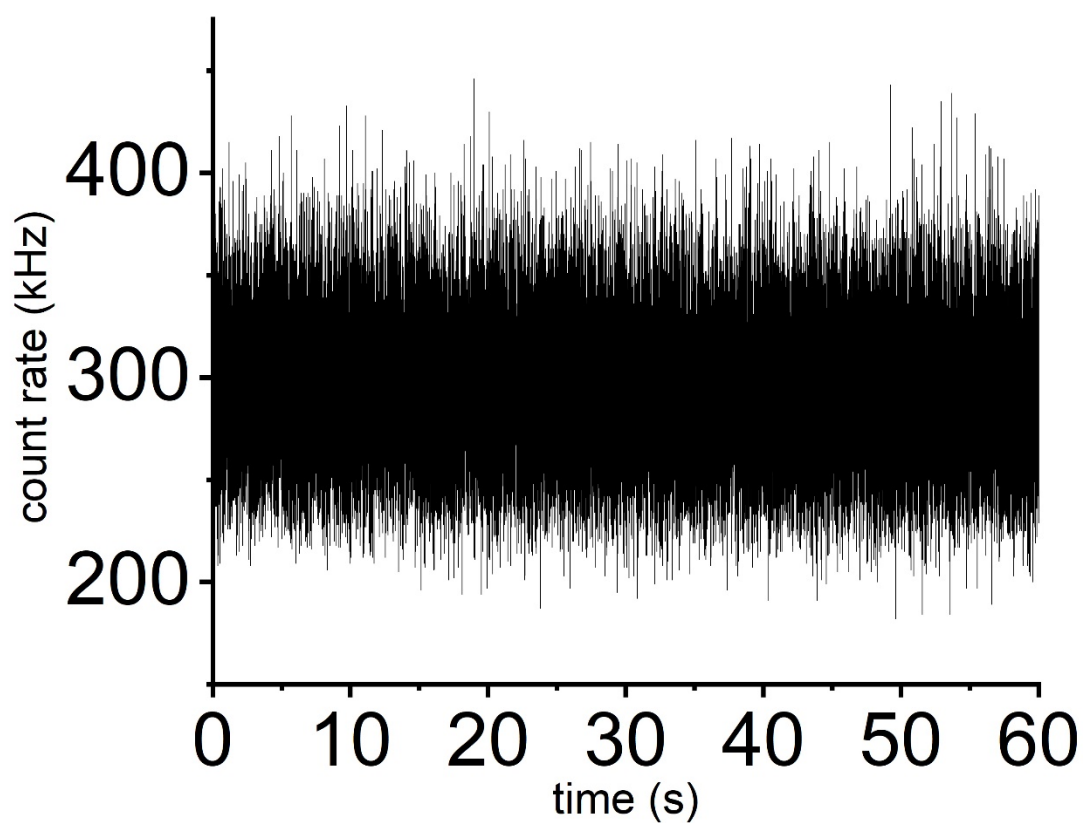


Figure S3. Intensity trace from a 60 s measurement on the same sample as in Figure S1. The lack of spikes in the intensity trace indicates that no large aggregates of A β 42 are present.

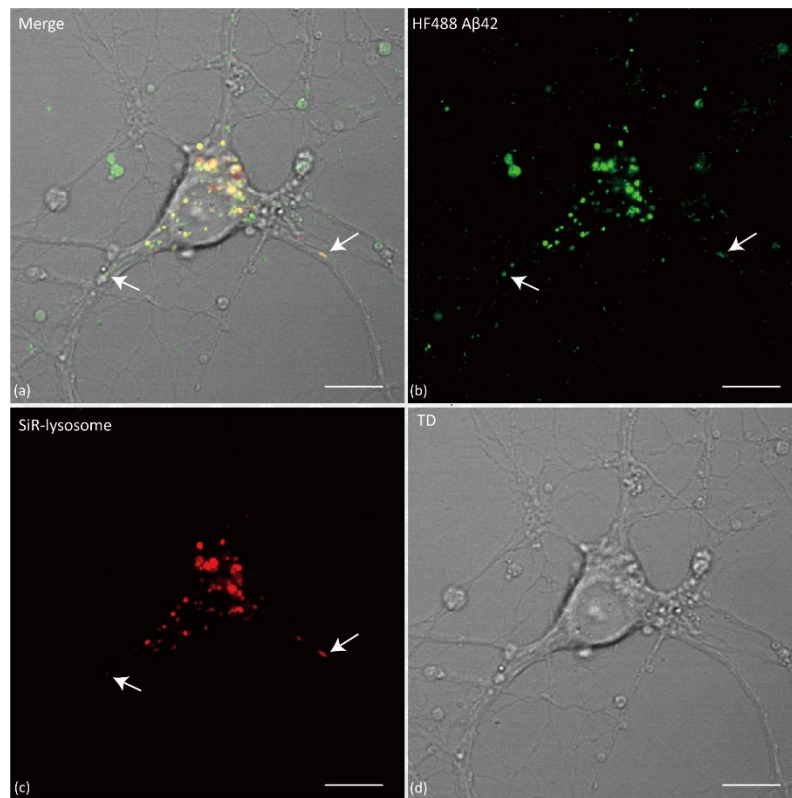


Figure S4. Confocal image of primary neurons (DIV21) treated with 1000 nM HF488 A β 42 for 24 hours. Most of A β 42 (green) were internalized into SiR-lysosome (red) positive vesicles in soma and a few A β 42 vesicles were in neurites (white arrow). After 24 hours treatment with A β , the morphology of neurons is not altered. (a) Merged channel; (b) HF488 A β 42; (c) SiR-lysosome; (d) Transmitted channel (TD). Scale bar: 10 μ m. The images were acquired by Nikon A1 Plus confocal with Plan Apo VC 60x Oil Objective (NA = 1.4). Pinhole size: 26.82 μ m. Pixel size: 0.11 μ m. HF-488 A β 42 channel, excitation: 488.0 nm, emission: 525.0 nm, GaAsP detector; SiR-lysosome channel, excitation: 640.8 nm, emission: 700.0 nm, PMT detector.

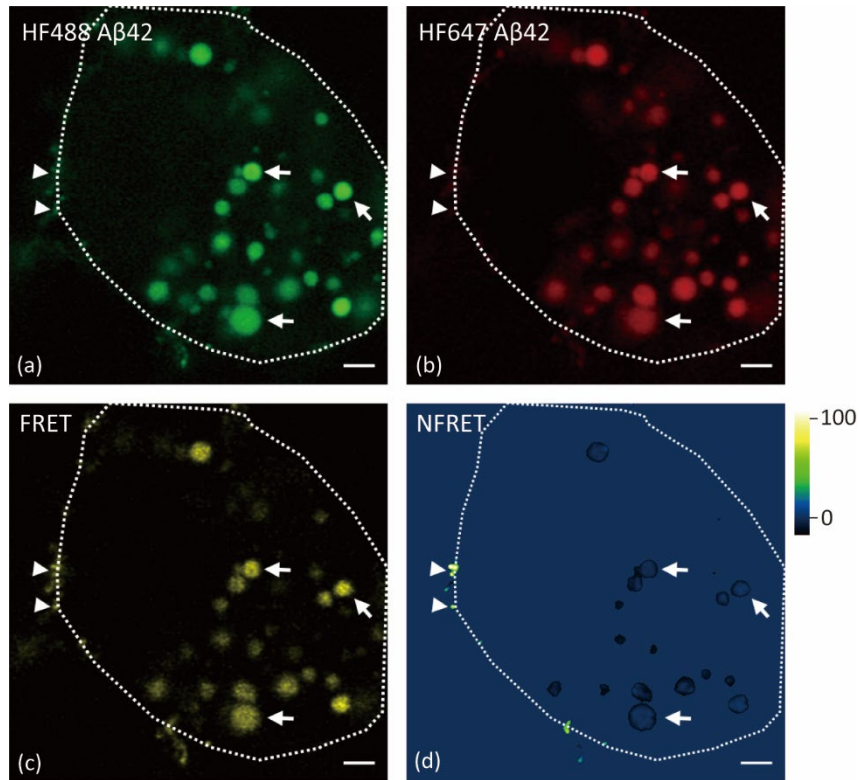


Figure S5. FRET images of A β on the cell surface. Primary neurons were treated with 125 nM HF488 A β 42 + 125 nM HF647 A β 42 for 24h. **(a)** HF488 A β 42 (Gamma = 0.45); **(b)** HF647 A β 42 (Gamma = 0.45); **(c)** FRET channel (Gamma = 1.0); **(d)** Normalized FRET (NFRET) image. The white arrow indicates the A β vesicles in soma, and the arrowhead indicates the A β on the cell surface. The cell body of neuron is outlined with the white dashed lines. Scale bar: 2 μ m.

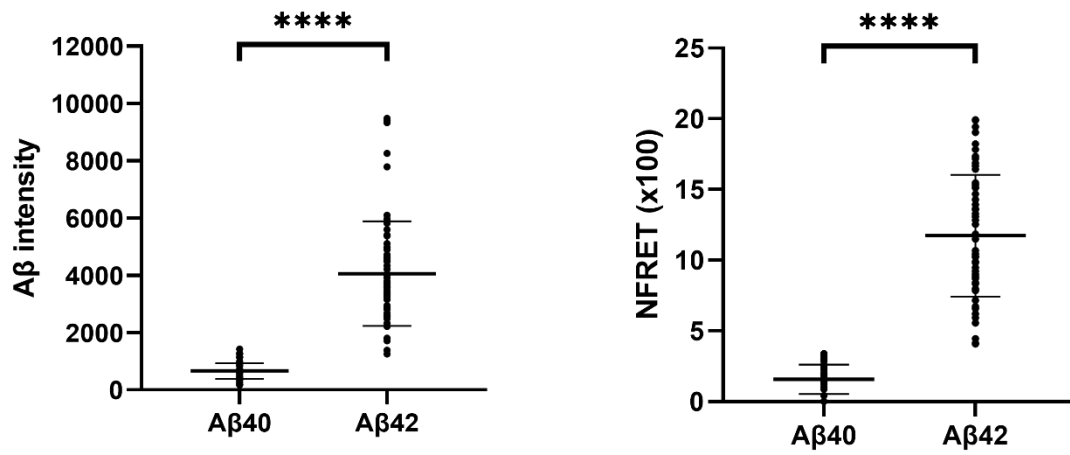


Figure S6. Comparison of primary neurons treated with 1000 nM A β 40 or A β 42 for 24h. A β intensity (HF647, left) and NFRET values (right) of A β vesicles in neurons treated with A β 40 (N=60, 1 cell per image from 8 images) and A β 42 (N=63, 1 cell per image from 8 images) were compared using Mann-Whitney test ($P < 0.0001$). Data are mean \pm S.D.