

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 $\tau_{D1} = 27 \mu\text{s}$ and $\tau_{D2} = 81 \mu\text{s}$ 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 $\tau_{D3} = 470 \mu\text{s}$. The slowest component indicates that a few oligomers of A β 42 are present.

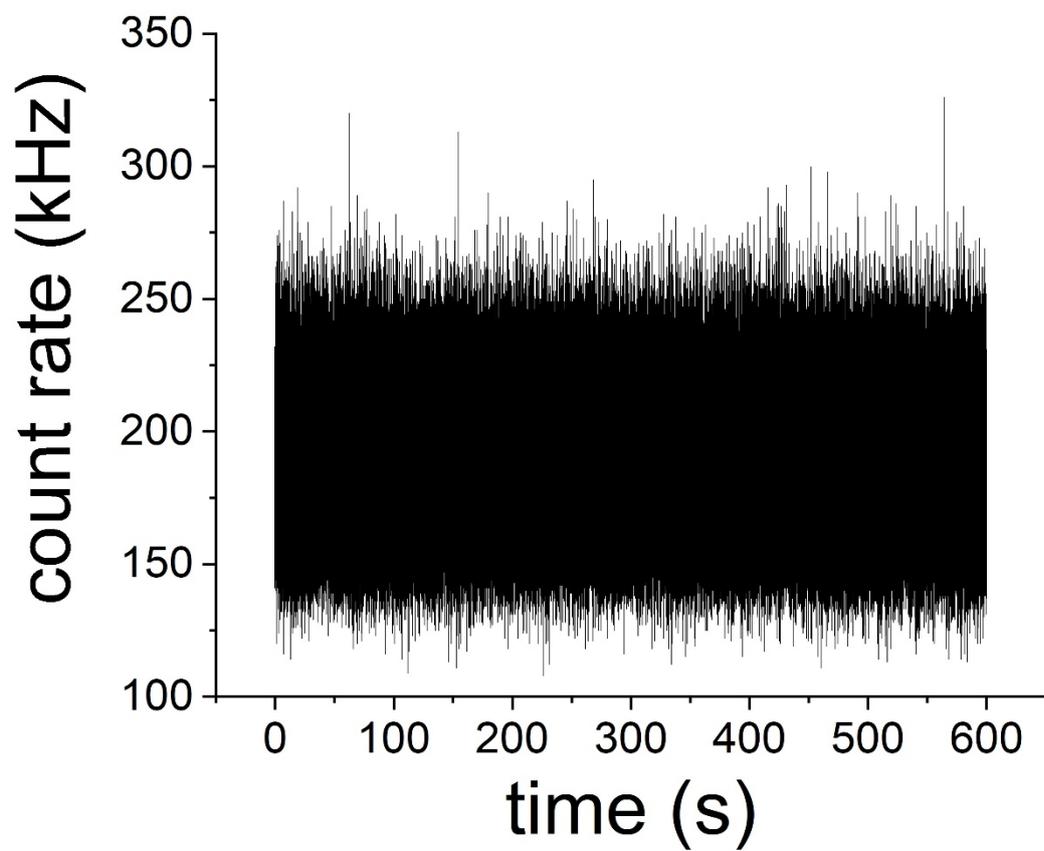


Figure S2. Intensity trace from a 10 min measurement on the same sample as in Figure S1. The lack of spikes in the intensity trace indicates that no large aggregates of A β 42 are present.

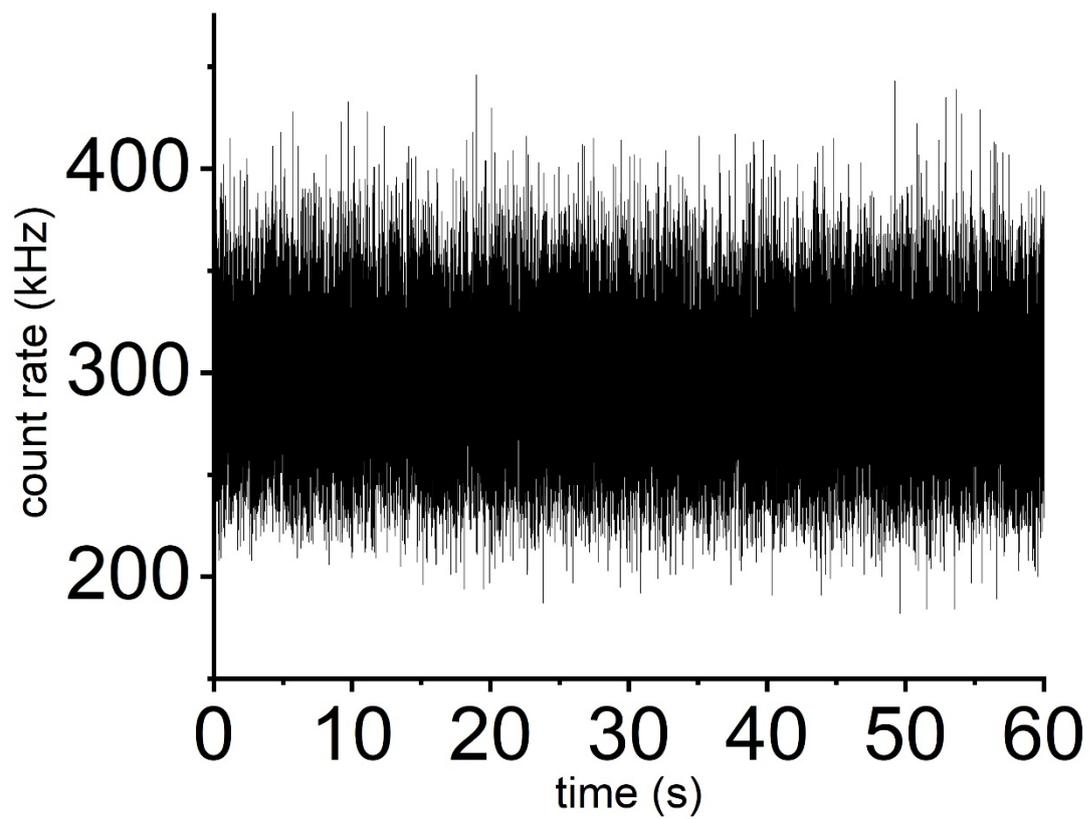


Figure S3. Intensity trace from a 60 s measurement on the same sample as in Figure S1. The lack of spikes in the intensity trace indicates that no large aggregates of A β 42 are present.

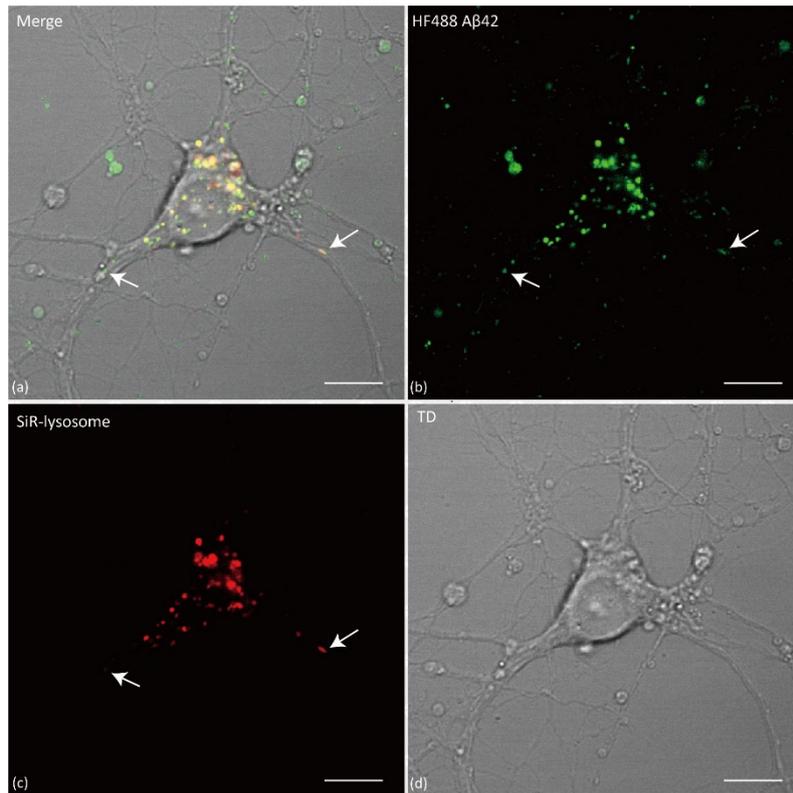


Figure S4. Confocal image of primary neurons (DIV21) treated with 1000 nM HF488 A β 42 for 24 hours. Most of A β 42 (green) were internalized into SiR-lysosome (red) positive vesicles in soma and a few A β 42 vesicles were in neurites (white arrow). After 24 hours treatment with A β , the morphology 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.

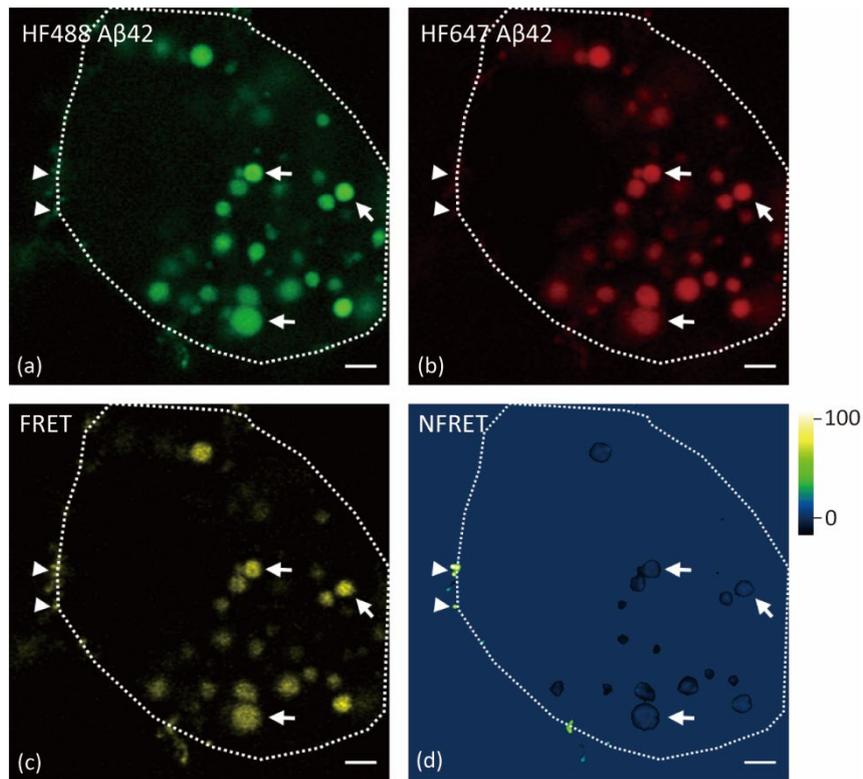


Figure S5. FRET images of A β on the cell surface. Primary neurons were treated with 125 nM HF488 A β 42 + 125 nM HF647 A β 42 for 24h. **(a)** HF488 A β 42 (Gamma = 0.45); **(b)** HF647 A β 42 (Gamma = 0.45); **(c)** FRET channel (Gamma = 1.0); **(d)** Normalized FRET (NFRET) image. The white arrow 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.

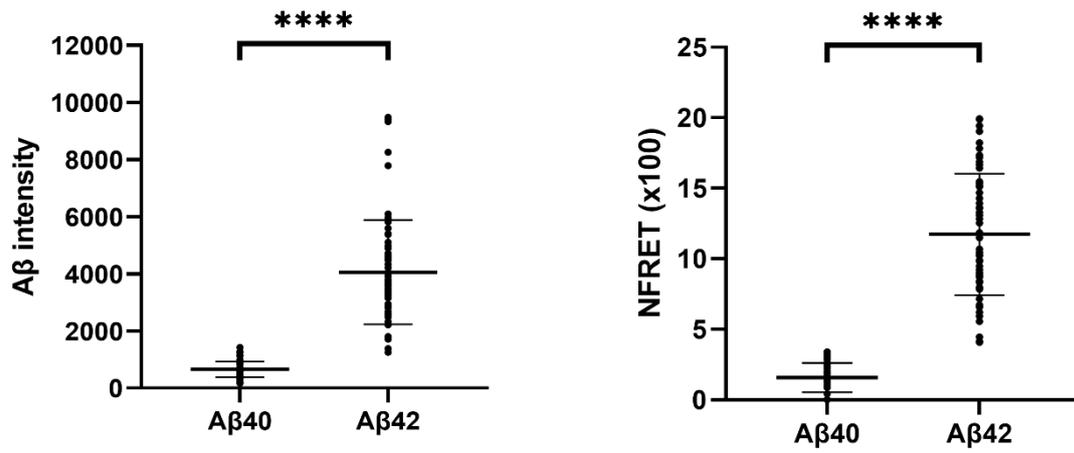


Figure S6. Comparison of primary neurons treated with 1000 nM Aβ40 or Aβ42 for 24h. Aβ intensity (HF647, left) and NFRET values (right) of Aβ vesicles in neurons treated with Aβ40 (N=60, 1 cell per image from 8 images) and Aβ42 (N=63, 1 cell per image from 8 images) were compared using Mann-Whitney test ($P < 0.0001$). Data are mean \pm S.D.