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Electronic supplementary information (ESI)

Apoferritin Fibers: a New Template for 1D Fluorescent Hybrid Nanostructures

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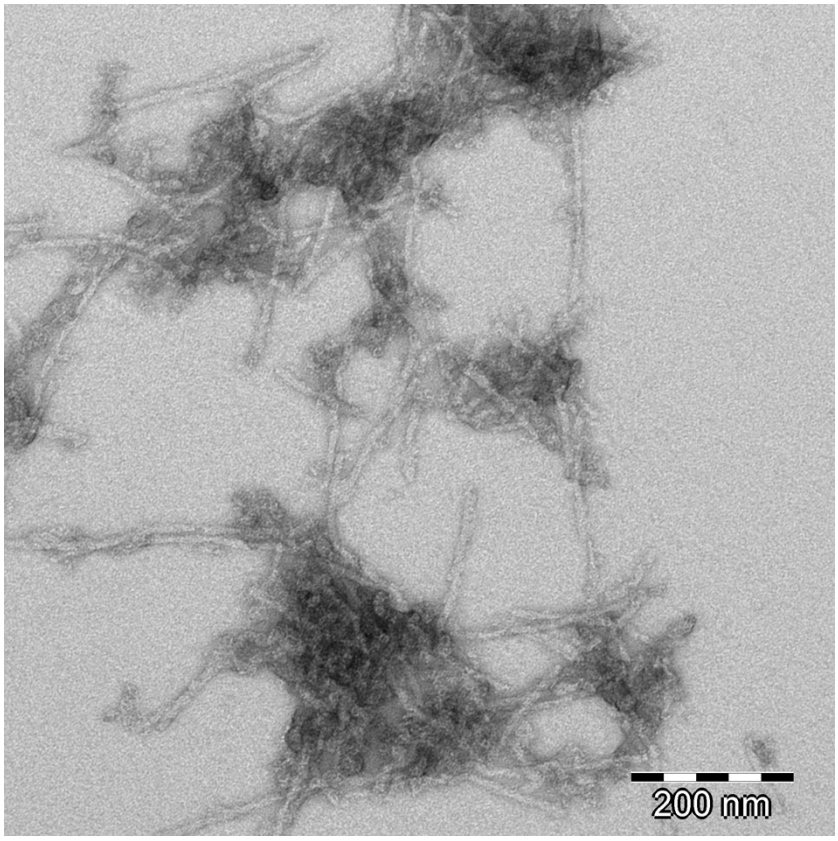

Figure S1. TEM image of ferritin protein n after heat treatment at 80ºC and pH 2 for 24h.

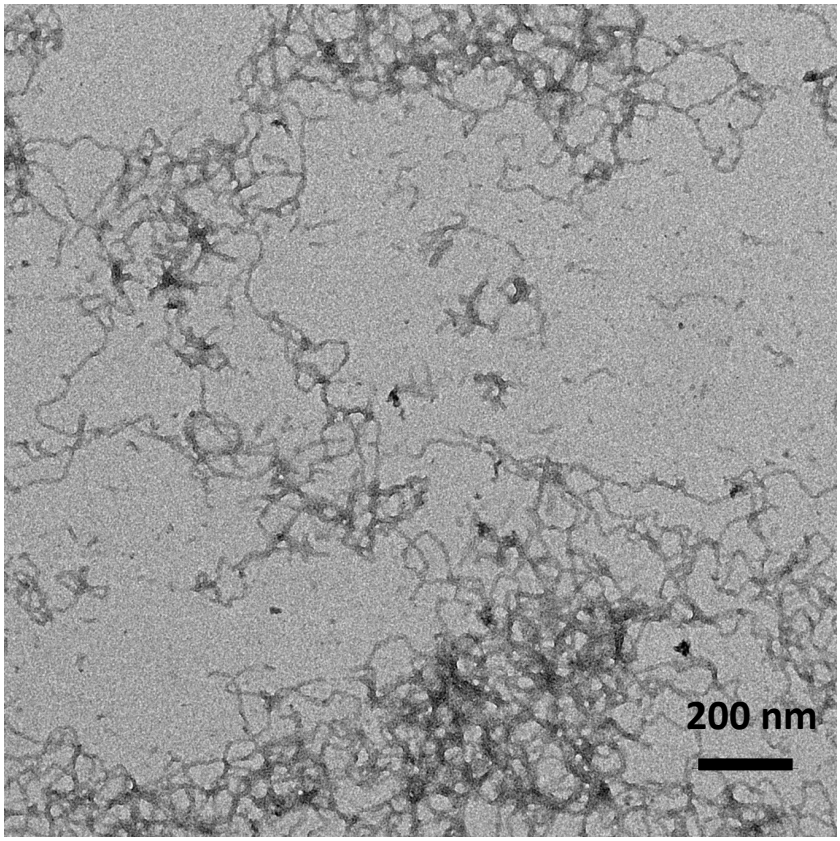

Figure S2. TEM image of APO protein after heat treatment at 50ºC and pH 5 for 18 days.

Fluorescence Lifetime Imaging Microscopy with Pulsed Interleaved Excitation (FLIM-PIE) Measurements.

For FLIM-PIE imaging collection, the APOft-fiber samples at concentration of 49 µM were diluted with Milli-Q water 300-fold to a final concentration of 163 nM; the APOft-globular samples, at 40 µM of protein concentration, were diluted 200-fold to a final concentration of 200 nM; the Blg-fibers at concentration of 67 µM were diluted 900-fold to a final concentration of 74 nM; and the Blg-globular samples at the concentration of 52 µM were diluted 2700-fold to a final concentration of 19 nM. After dilution, 40 µL of sample were deposited on a microscope cover slide (Thermo-Scientific, Menzel-Gläser, Brawnschweig, Germany) previously washed 5x with milli-Q water.

FLIM-PIE experiments were performed with a MicroTime 200 time-resolved fluorescence microscope (PicoQuant GmbH, Germany). We employed a dual-color pulsed interleaved excitation (PIE) scheme, with two spatially overlapped pulsed lasers, at 470 (LDH-P-C-470, PicoQuant) and 635 nm (LDH-P-635, PicoQuant). The total excitation pulse frequency was 20 MHz, with the 635-nm laser delayed 30 ns (Ortec delay box) with respect the 470-nm laser pulse. By applying specific time windows (Fig. S4), these settings permit one-step reconstruction of the donor dye FLIM image, FRET fluorescence image, and directly excited acceptor FLIM image which make FLIM-PIE an extremely suited tool for quantitatively measuring FRET between the two different dyes. The excitation power of each laser at the microscope entrance was adapted at the emission properties of the proteins analyzed. For the FLIM measurements of the

APOft-fibers, the excitation power was 448 nW for the 470-nm and 24 nW for the 635-nm laser. For the APOft-globular, the power was 980 nW for the 470-nm and 178 nW for the 635-nm laser. For the Blg-fiber the excitation power was 372 nW and 23 nW for the 470-nm and the 635-nm laser, respectively. Finally, for the Blg-globular experiments, the laser power was 1.88 μW and 51.5 nW for the 470-nm and the 635-nm laser, respectively.

The excitation beams were focused just above the glass surface, and the fluorescence emission was collected through a high numerical aperture oil-immersion objective (Plan Achromat 100x/1.40, Olympus), and then imaged onto a 75-μm pinhole (Melles Griot). The transmitted signal was then separated by a 600 dcxr dichroic beam splitter (AHF/Chroma), filtered into the donor (520/35 Omega Filters) and acceptor (685/70 Omega Filters) channels, and directed to two avalanche photodiodes (APD) (SPCM-AQR-14, Perkin-Elmer Optoelectronics). FLIM images were collected with a time resolution of 116 ps per channel, an image size of 15 μM x 15 μM, a pixel resolution of 512 × 512 and a collection time of 0.60 ms per pixel.

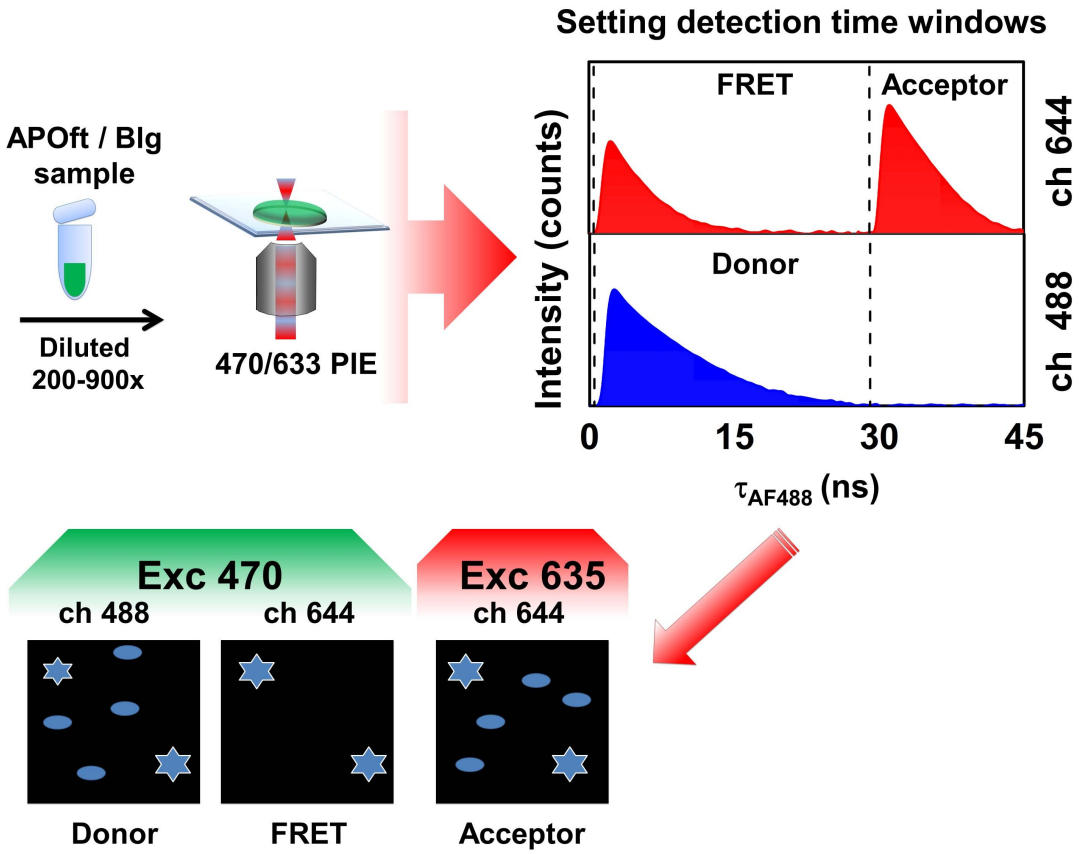

Figure S3. FLIM-PIE scheme experiment.

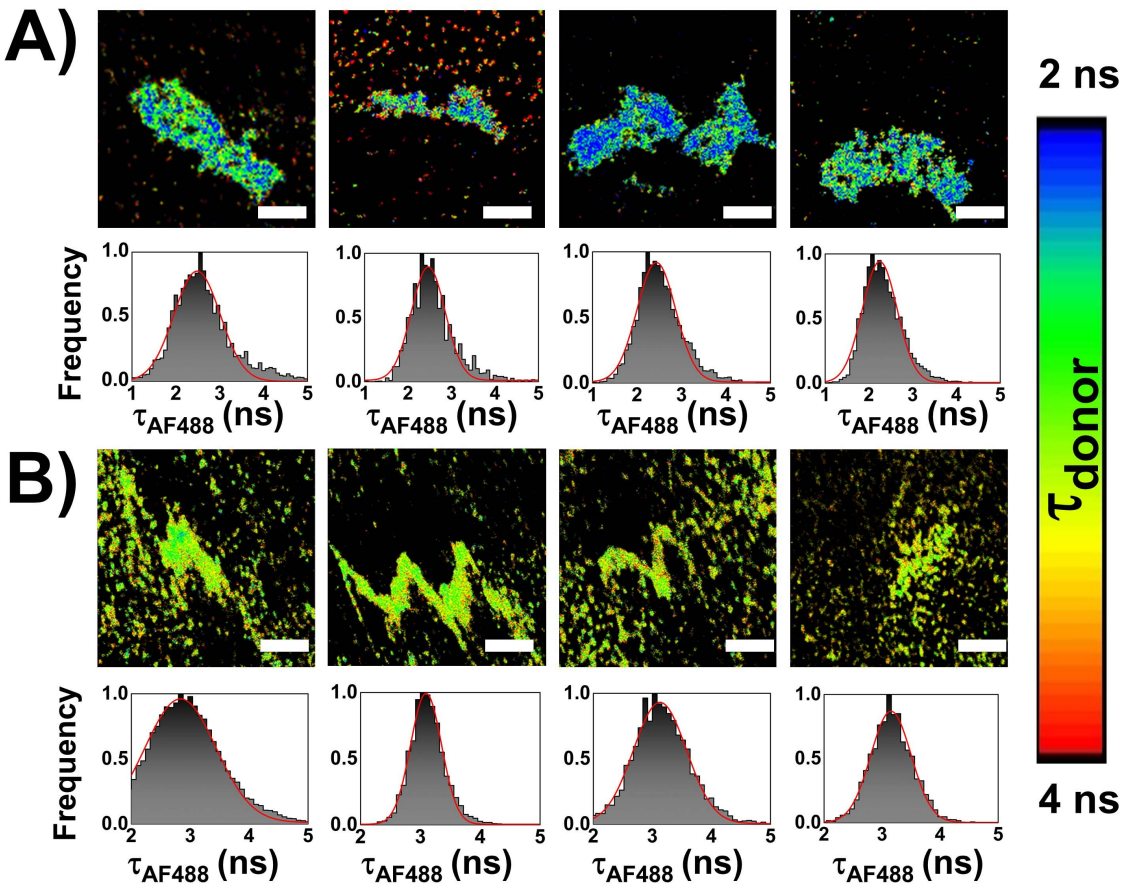

Figure S4. Donor FLIM images. A: APOft-fibers and the corresponding donor lifetime distributions of the smaller aggregates. B: Blg-fibers and the corresponding donor lifetime distributions of the smaller aggregates. The images are represented in a pseudo-color scale from 2 to 4 ns. The scale bars represent 3.3 µm.

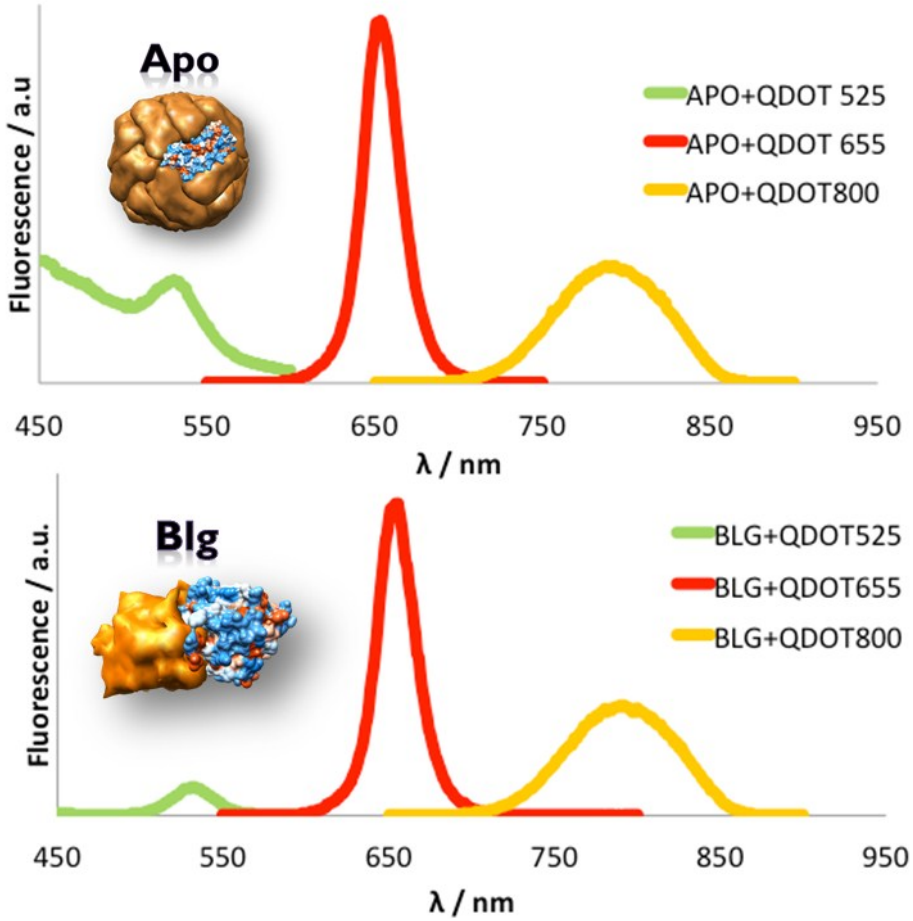

Figure S5. Fluorescence spectra of up: APO-QD525, APO-QD655 and APO-QD800, and bottom: Blg-QD525, Blg-QD655 and Blg-QD800 hybrid fibers under excitation at 350 nm.