

Electronic Supplementary Information for  
**Specific Binding and Internalization:  
An Investigation of Fluorescent Aptamer-Gold Nanoclusters and Cells  
with Fluorescence Lifetime Imaging Microscopy**

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**Physical properties of non-biofunctionalized 11-MUA-AuNCs**

Figure S1 shows (a) a TEM image with a histogram displaying the particle size distribution and (b) UV-Vis absorption and emission spectra of freshly synthesized, non-biofunctionalized 11-MUA-AuNCs. Figure S1 (c) shows the result of the viability assay, which shows a good biocompatibility for the 11-MUA-AuNCs.

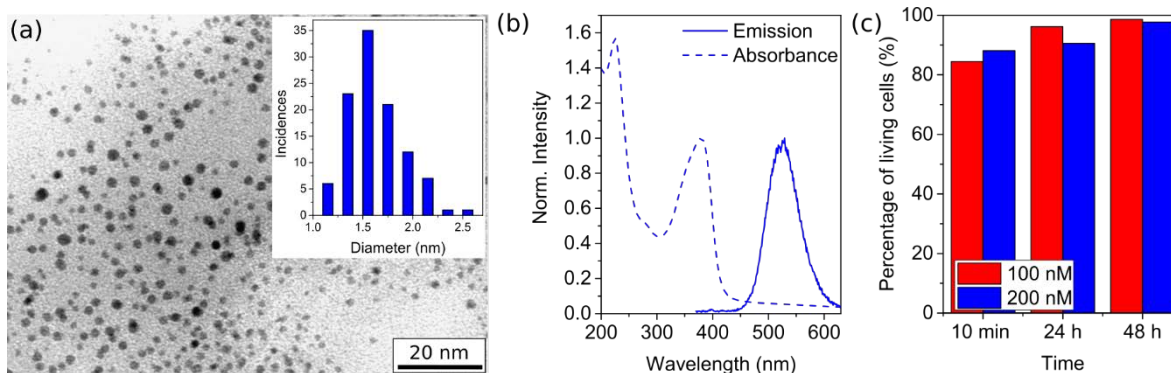


Figure S1: (a) TEM image with a histogram containing the particle size distribution, (b) UV-Vis spectra, and (c) cell viability assay of 11-MUA-AuNCs.

## Physical properties of BaF3 cells

Figure S2 shows (a) a dark field light microscope image, (b) an SEM image, and (c) an AFM line-scan of BaF3 cells. To verify the expression of the specifically addressed Interleukin-6-receptor, we performed flow cytometry assays with ATTO633-labeled aptamer AIR-3A. For the flow cytometry assay we followed the protocol by Meyer and coworkers.<sup>29</sup> Figure S2 (d) gives corresponding results.

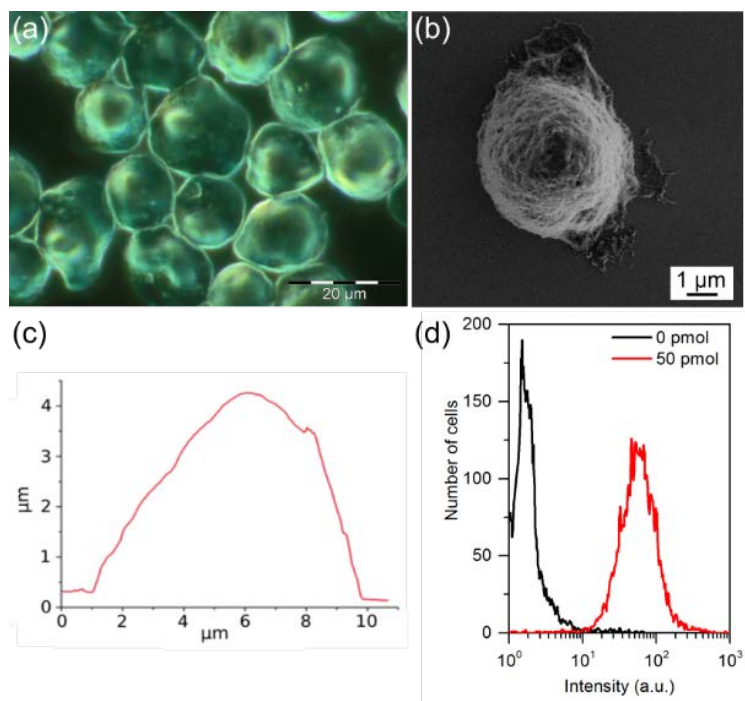


Figure S2: (a) Dark field image, (b) SEM image, and (c) AFM line scan of a dried BaF3 cell. (d) Flow cytometry plot of cells incubated with 0 and 50 pmol ATTO633-AIR-3A.

## FLIM images after the first incubation step

Figure S3 shows the complete set of FLIM images of all  $z$  heights (0.0 – 4.5  $\mu\text{m}$ ) after the first incubation step of a BaF3 cells incubated with aptamer-AuNCs. The particular timeframes for the gated FLIM images and the time and intensity scaling for direct FLIM images were chosen to be the same as for Figure 2 (c) and (d).

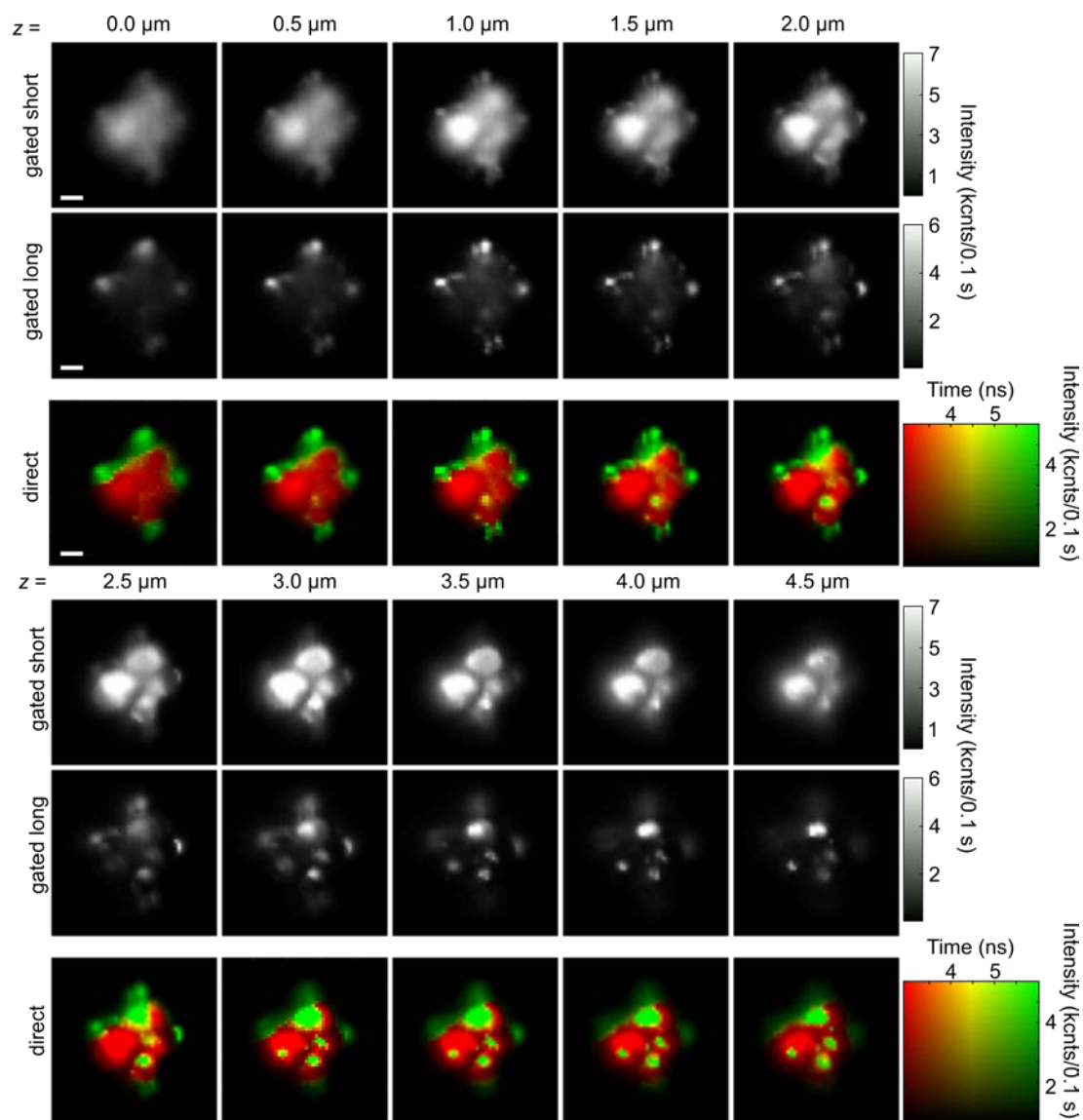


Figure S3: Gated and direct FLIM images of all  $z$  heights (0.0 – 4.5  $\mu\text{m}$ ) of a BaF3 cell incubated with aptamer-AuNCs for 10 min at 37  $^{\circ}\text{C}$  corresponding to figure 2. Scale bar 2  $\mu\text{m}$ .

### FLIM images of a BaF3 cell without AuNCs

The FLIM data of a BaF3 cells incubated without adding AuNCs was analyzed in the same way as explained in the main text. All  $z$  heights, the particular timeframes for the gated FLIM images, and the time and intensity scaling for direct FLIM images were chosen to be the same as for Figure 2 (c,d) as well as Figure 3(b,c).

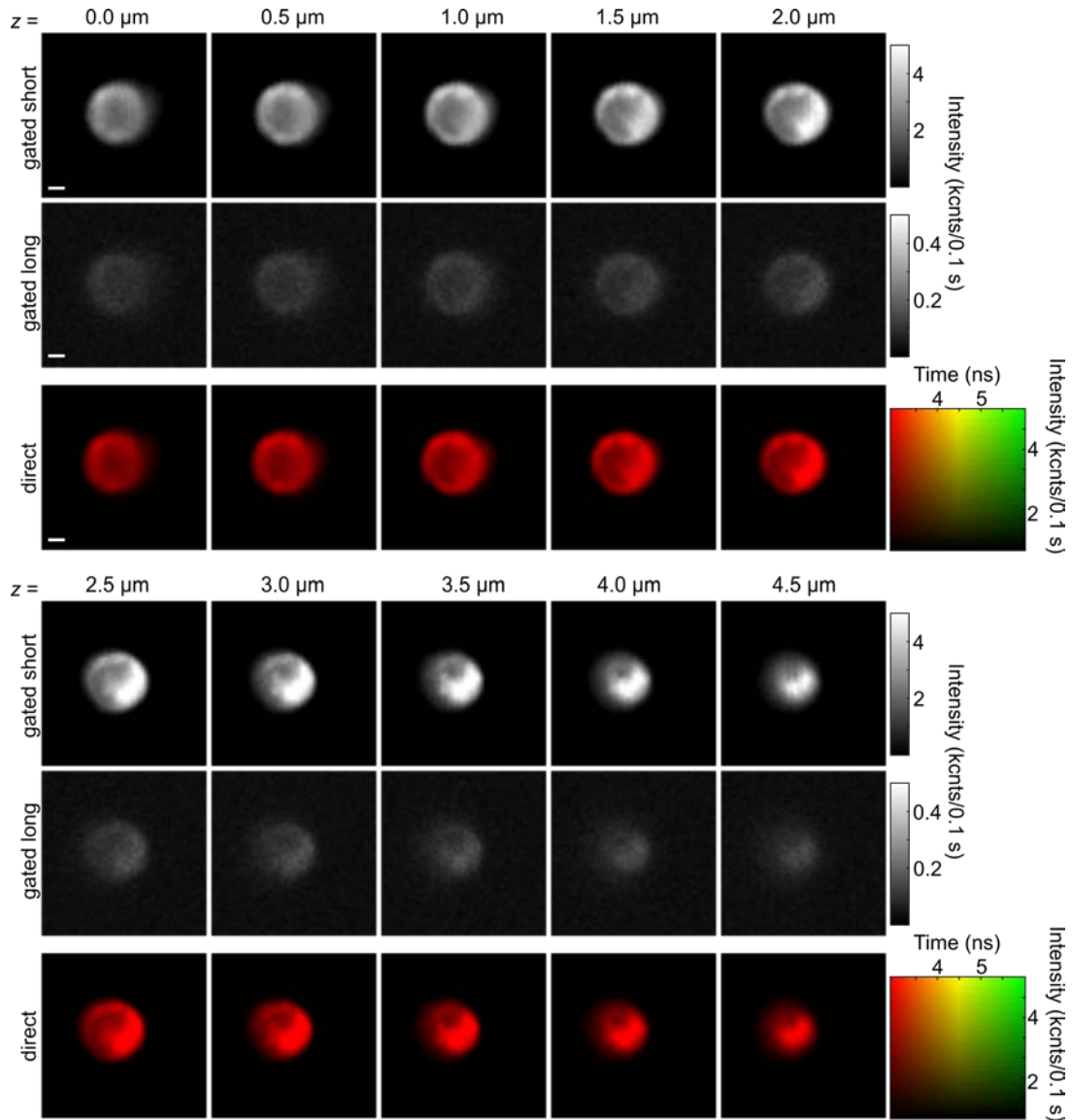


Figure S4: Gated and direct FLIM images of a BaF3 cell without any AuNCs. Scale bar 2  $\mu\text{m}$ .

## FLIM images of a BaF3 cell incubated with 11-MUA-AuNCs

Figure S5 shows FLIM images after the first incubation step of two BaF3 cells incubated with non-biofunctionalized 11-MUA-AuNCs. The particular timeframes for the gated FLIM images and the time scaling for direct FLIM images were chosen to be the same as for Figure 2 (c,d) and Figure 3 (b,c).

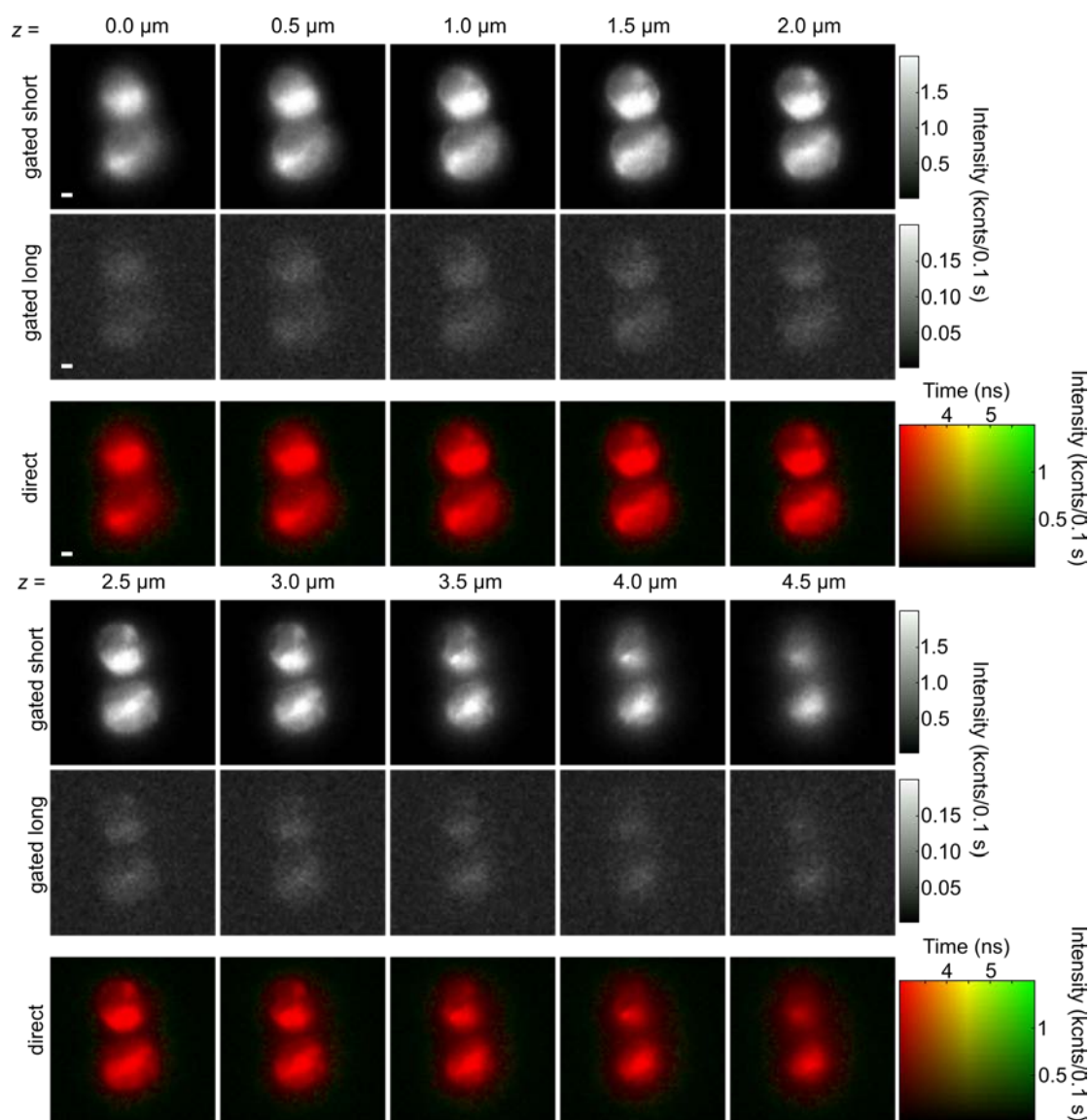


Figure S5: FLIM images of two BaF3 cells incubated with 11-MUA-AuNCs for 10 min at 37 °C. Scale bar 2  $\mu\text{m}$ .

## FLIM images of a PC3 cell incubated with aptamer-AuNCs

Figure S6 shows FLIM images after the first incubation step of a PC3 cells incubated with biofunctionalized aptamer-AuNCs. All  $z$  heights, the particular timeframes for the gated FLIM images, and the time and intensity scaling for direct FLIM images were chosen to be the same as for Figure 2 (c,d) as well as Figure 3 (b,c).

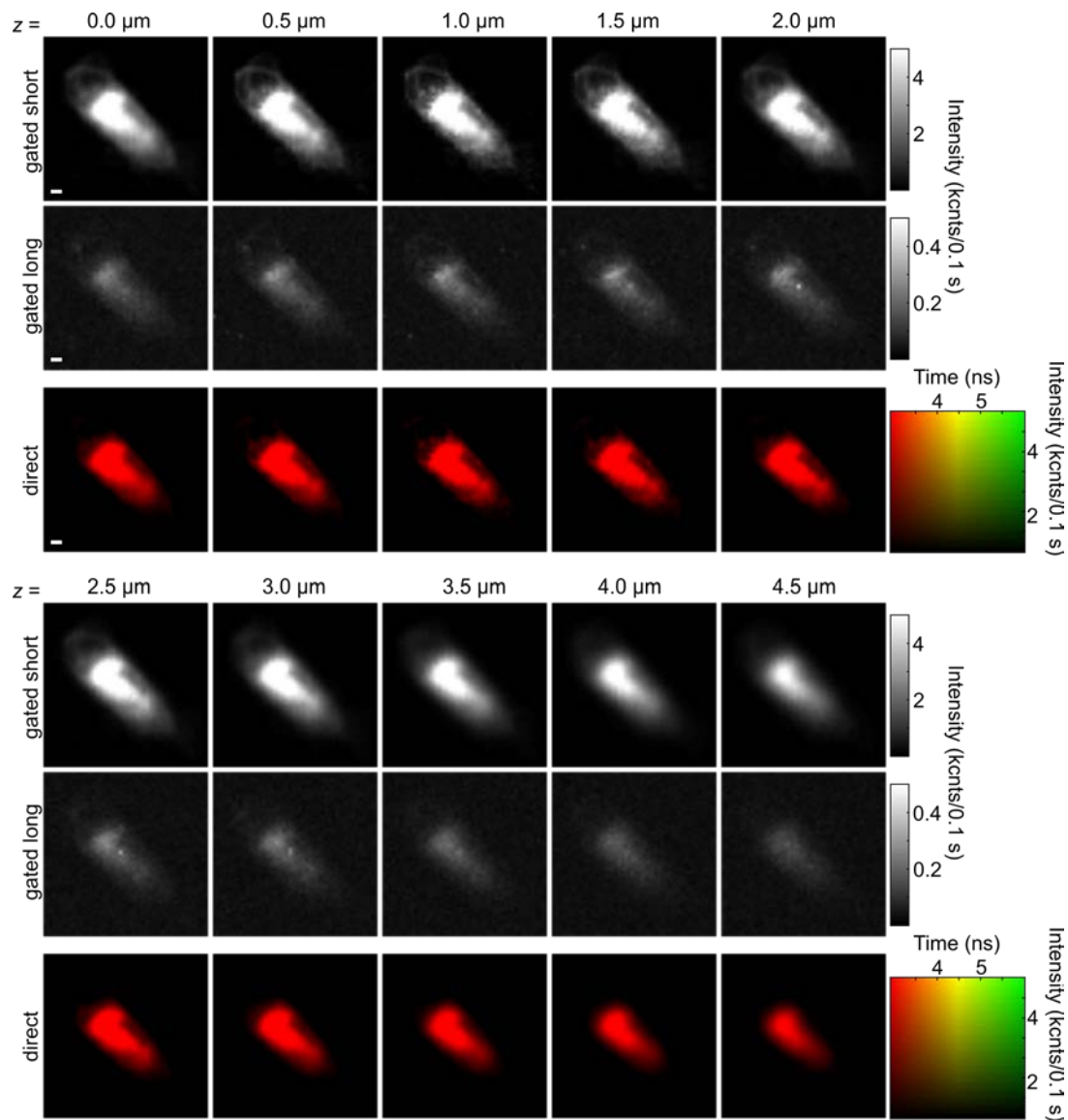


Figure S6: FLIM images of a PC3 cell incubated with aptamer-AuNCs for 10 min at 37 °C. Scale bar 2  $\mu\text{m}$ .



## Correlated photoluminescence/SEM/AFM measurements

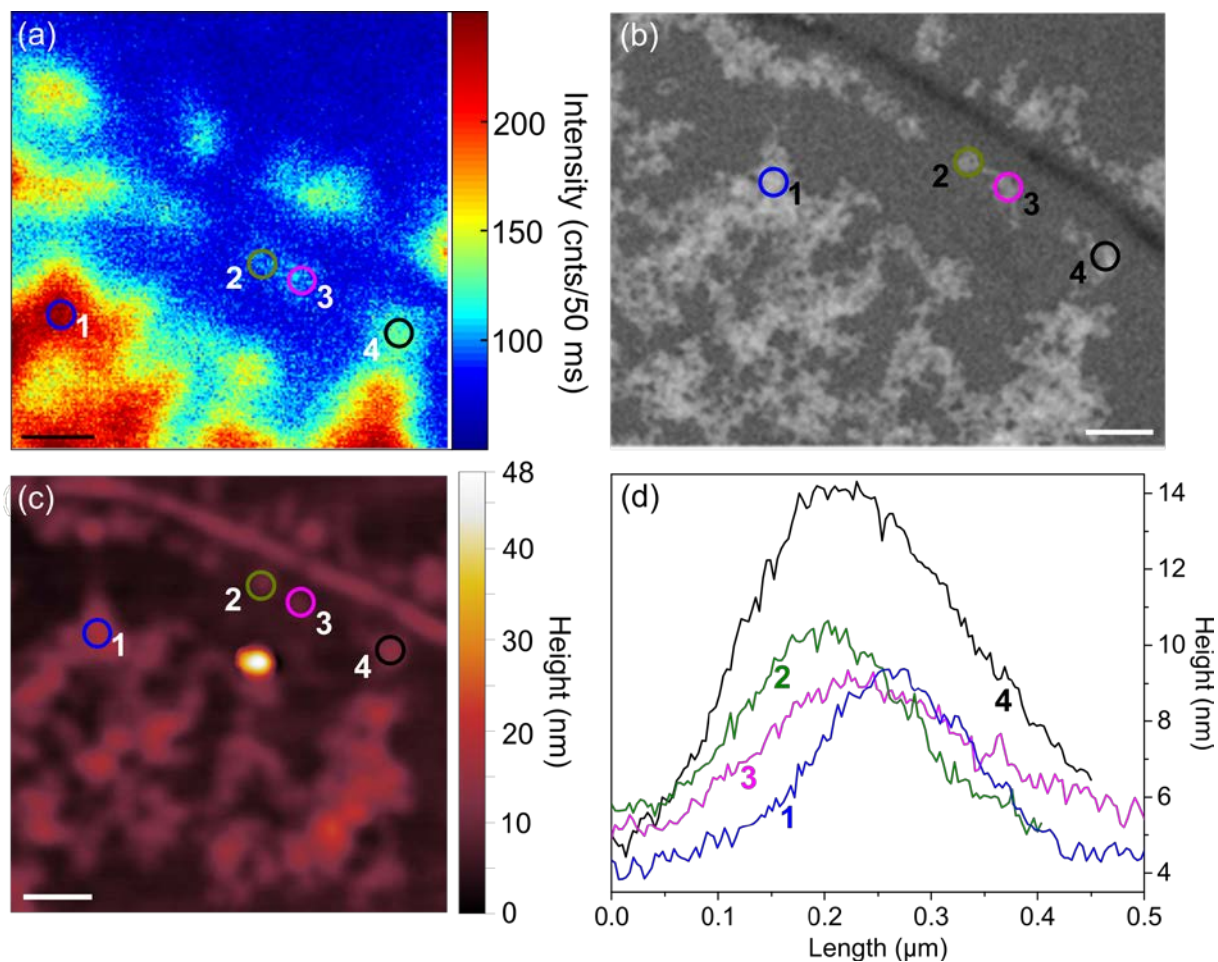


Figure S7: Correlated (a) photoluminescence, (b) element specific SEM, and (c) AFM images. (d) AFM cross sections of the encircled areas of 11-MUA-AuNCs. Scale bar: 500 nm.

Tabel S1: Correlated results of photoluminescence, element specific SEM, and AFM measurements.

Circle	Intensity (cnts/50 ms)	Area SEM (nm <sup>2</sup> )	AFM Height (ns)	Particle (#)
1	~250	-	5	-
2	80-110	7482	5	470
3	80-120	11689	4	735
4	~180	52380	9	3294

## FLIM images after the second incubation step

Figure S8 shows the complete set of FLIM images of all  $z$  heights (0.0 – 4.5  $\mu\text{m}$ ) after the second incubation step. The particular timeframes for the gated FLIM images and the time and intensity scaling for direct FLIM images were chosen to be the same as for Figure 3(b) and (c).

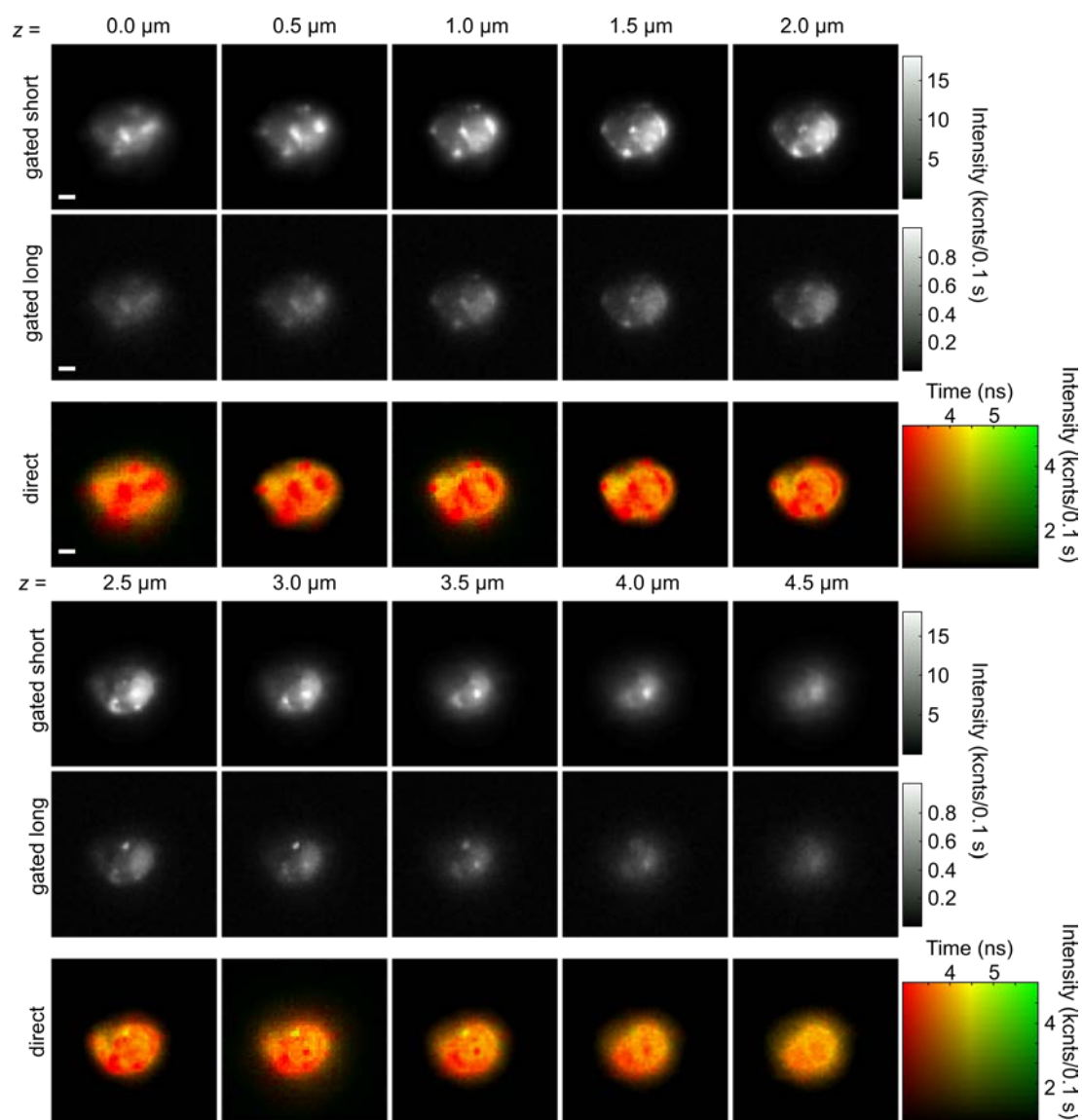


Figure S8: Gated and Direct FLIM images of all  $z$  heights (0.0 – 4.5  $\mu\text{m}$ ) of a BaF3 cells incubated with aptamer-AuNCs for 10 min at 37  $^{\circ}\text{C}$  and again for 10 min at 37  $^{\circ}\text{C}$  after washing corresponding to figure 3. Scale bar 2  $\mu\text{m}$ .



### pH influence on the fluorescence decay of 11-MUA-AuNCs

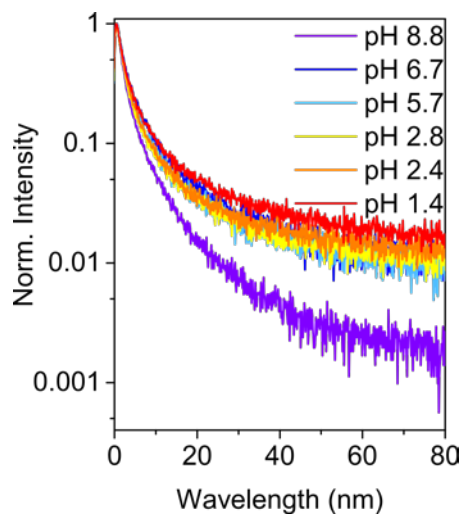


Figure S9: Normalized fluorescence decay curves of purified 11-MUA-AuNCs at pH values ranging from 8.8 to 1.4.