

Supporting Information

pH-Responsive Polyelectrolyte Complexation on Upconversion Nanoparticles: A Multifunctional Nanocarrier for Protection, Delivery, and 3D-Imaging of Therapeutic Protein

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1. Energy-Dispersive X-ray Spectroscopy

Table S1. Distribution of lanthanide ions in the core-shell nanoparticles determined by energy-dispersive X-ray spectroscopy.

Y (wt%)	Yb (wt%)	Er (wt%)
90.01	9.16	0.92

2. Estimation of the Concentration of BSA Loaded on UCNP functionalized by poly-D-lysine (ULB) and poly-acrylic acid (UAB) *via* UV-vis Spectroscopy

Equation obtained from Figure 4b

$$Y = 0.012 + 0.004 X \quad \dots \quad (1)$$

Amount of BSA in the Supernatant obtained from UAB sample,

$$\begin{aligned} X &= (Y - 0.012) / 0.004 \\ &= (0.17159 - 0.012) / 0.004 \\ &= 39.8 \end{aligned}$$

Amount of BSA in the Supernatant obtained from ULB sample,

$$\begin{aligned} X &= (Y - 0.012) / 0.004 \\ &= (0.12284 - 0.012) / 0.004 \\ &= 27.7 \end{aligned}$$

Result:

Amount of loaded BSA in UAB = $(100 - 39.8) \mu\text{M} = 60.2 \mu\text{M}$ (~60%)

Amount of loaded BSA in ULB = $(100 - 27.7) \mu\text{M} = 72.3 \mu\text{M}$ (~72%)

3. Investigation of the Internalization Process of BSA loaded functionalized UCNPs and the release of BSA in HeLa Cells *via* live-cell imaging

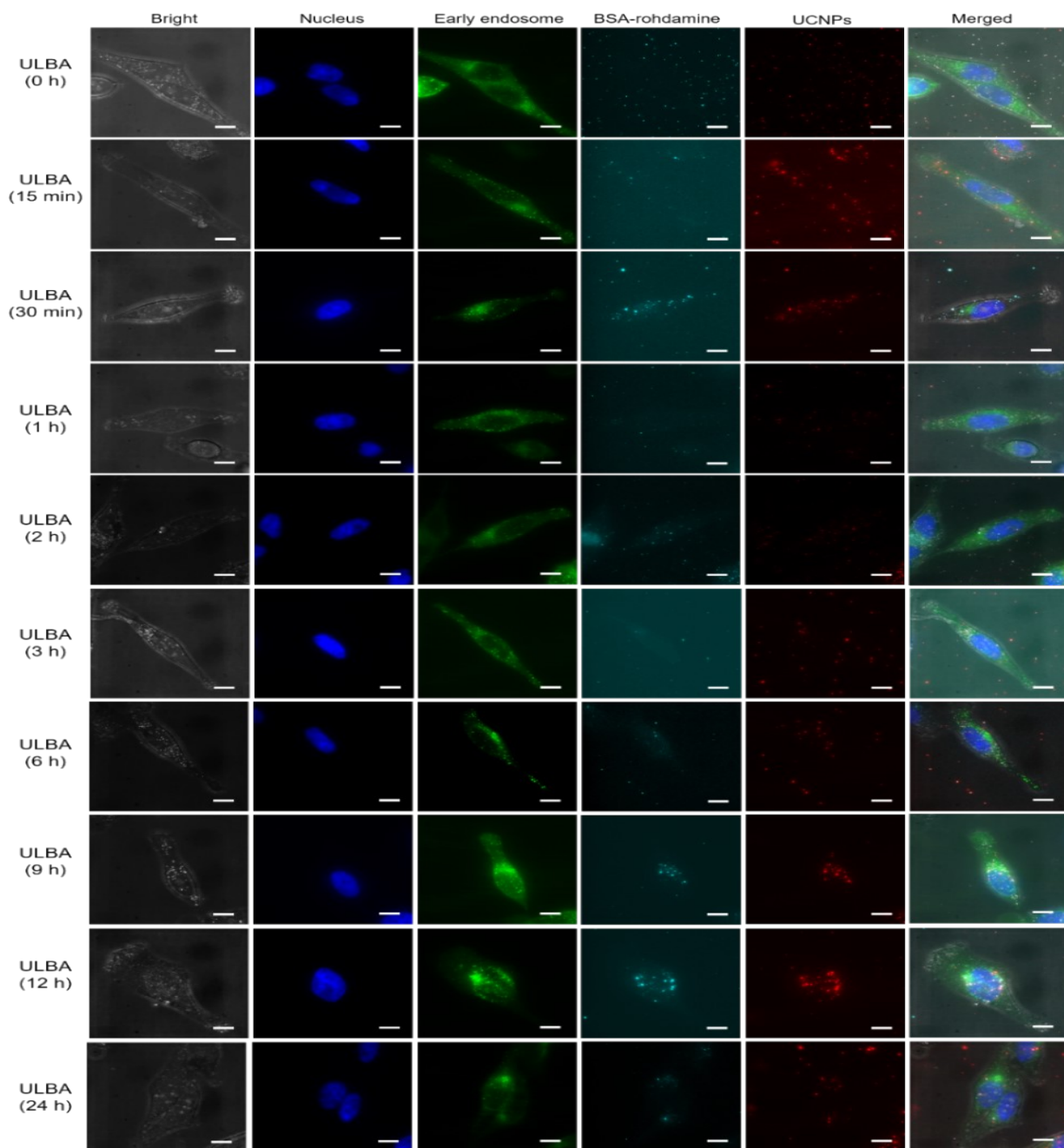


Figure S1. Results of 2D imaging for co-localization test of UCNP@Polylysine@BSA@Polyacrylic acid (ULBA) with early endosome stained HeLa cells. Cell (grey), nucleus (blue), early endosome (green), BSA-rhodamine (cyan), UCNPs (red) and merged images at each time point. Scale bar = 10 μ m

4. Investigation of the Internalization Process of BSA loaded functionalized UCNPs and the release of BSA in HeLa Cells *via* 3D live-cell imaging

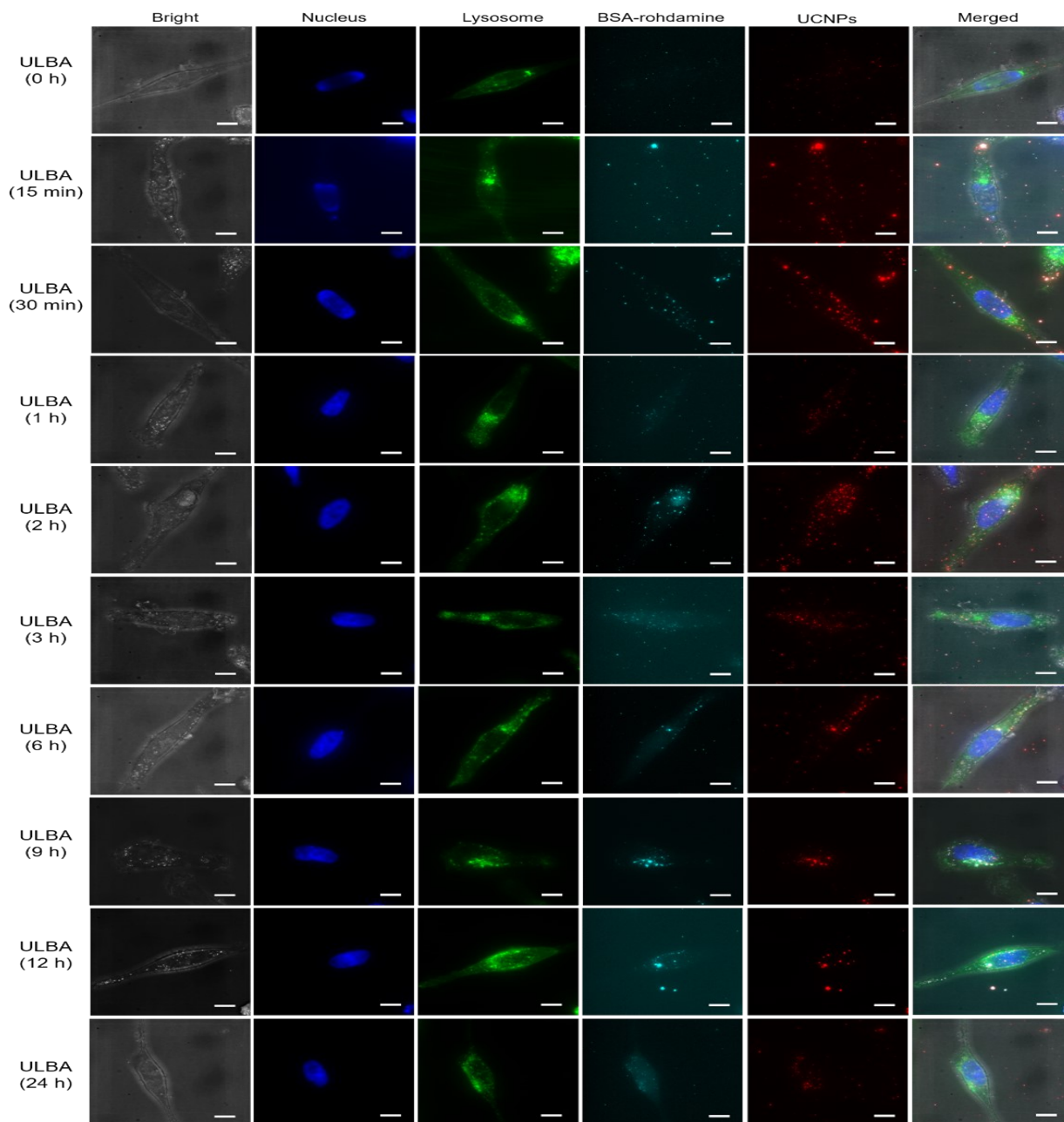


Figure S2. Results of 2D imaging for co-localization test with lysosome stained HeLa cells. cell (grey), nucleus (blue), lysosome (green), BSA-rhodamine (cyan), UCNPs (red) and merged images at each time point. Scale bar = 10 μ m

Note: The movie captions are listed below, and the movie files are uploaded separately.

List of Movie Captions

Movie S1. Early endosome at 0 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped on bringing the cursor on image).

Movie S2. Early endosome at 15 min post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S3. Early endosome at 30 min post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S4. Early endosome at 1 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S5. Early endosome at 2 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S6. Early endosome at 3 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S7. Early endosome at 6 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S8. Early endosome at 9 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the 'play' icon which gets popped up on bringing the cursor on image).

Movie S9. Early endosome at 12 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S10. Early endosome at 24 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S11. Lysosome at 0 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S12. Lysosome at 15 min post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S13. Lysosome at 30 min post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S14. Lysosome at 1 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S15. Lysosome at 2 h post internalization. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S16. Lysosome at 3 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S17. Lysosome at 6 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S18. Lysosome at 6 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S19. Lysosome at 12 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

Movie S20. Lysosome at 24 h post internalization of ULBA. Color code: Green: Early endosome; Cyan: BSA-Rhodamine; Red: UCNPs; Blue: Nucleus. (To watch video, click at the ‘play’ icon which gets popped up on bringing the cursor on image).

References

- [1] S.F. Himmelstoß, T. Hirsch, Long-Term Colloidal and Chemical Stability in Aqueous Media of NaYF₄-Type Upconversion Nanoparticles Modified by Ligand-Exchange, Part. Part. Syst. Charact. 36 (2019) 1900235. <https://doi.org/10.1002/ppsc.201900235>.
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