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Supporting Information

for *Small.,* DOI: 10.1002/smll.201502171

Ratiometric Organic Fibers for Localized and Reversible Ion Sensing with Micrometer-Scale Spatial Resolution

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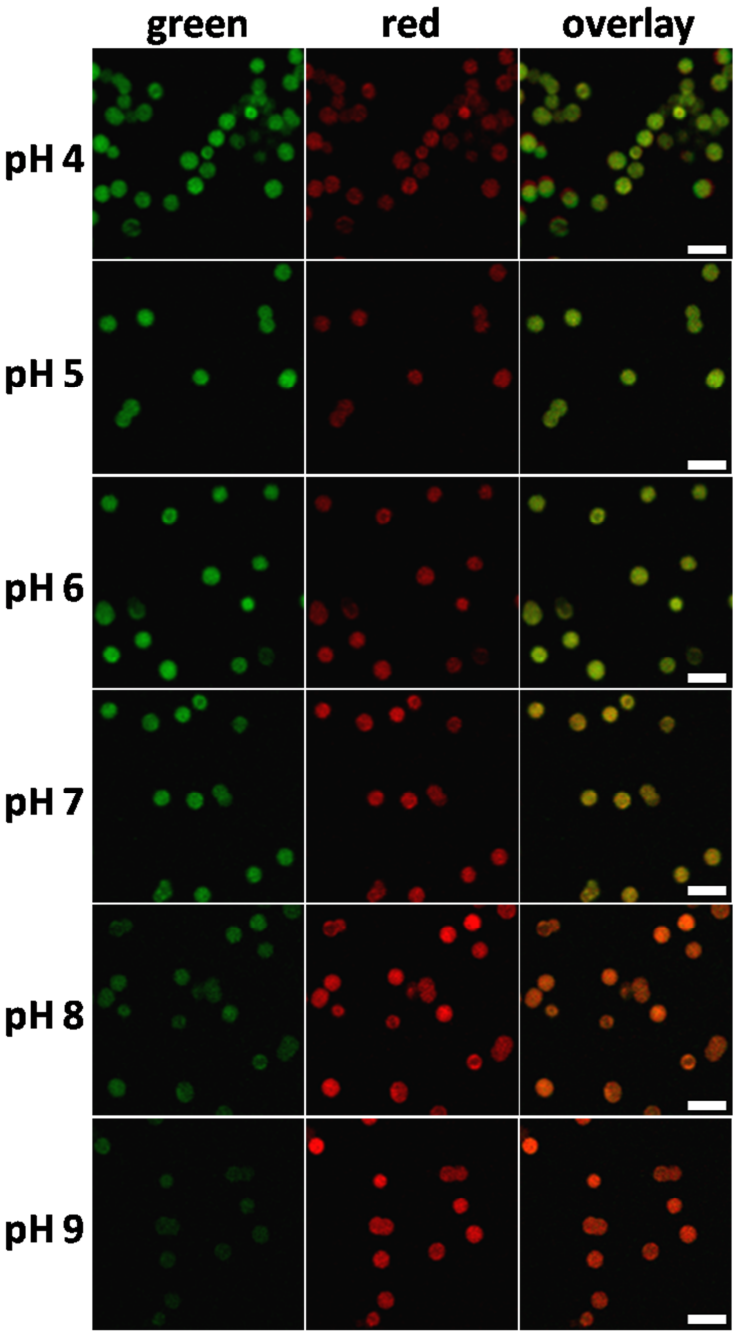

Figure S1. Fluorescent micrographs of capsule-based pH sensors at different pHs. The individual green (false color, 540-610 nm) and red (620-700 nm) are shown, followed by overlay of the two channels. Scale bars = 5 μm.

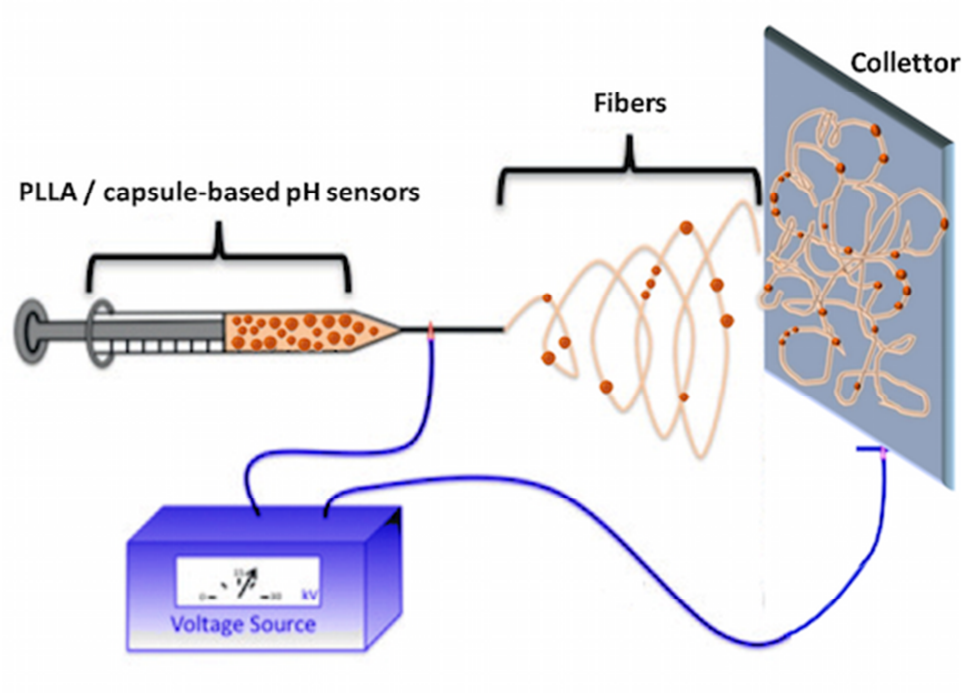

Figure S2. Schematic drawing of the electrospinning setup.

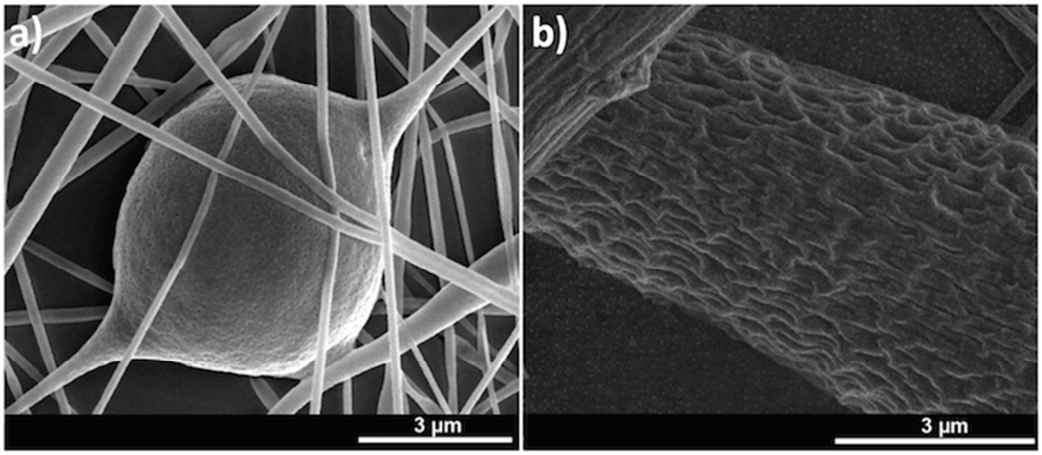

Figure S3. SEM micrographs showing the fiber porosity in the sensing region (a) and the roughness on the body of individual wires (b).

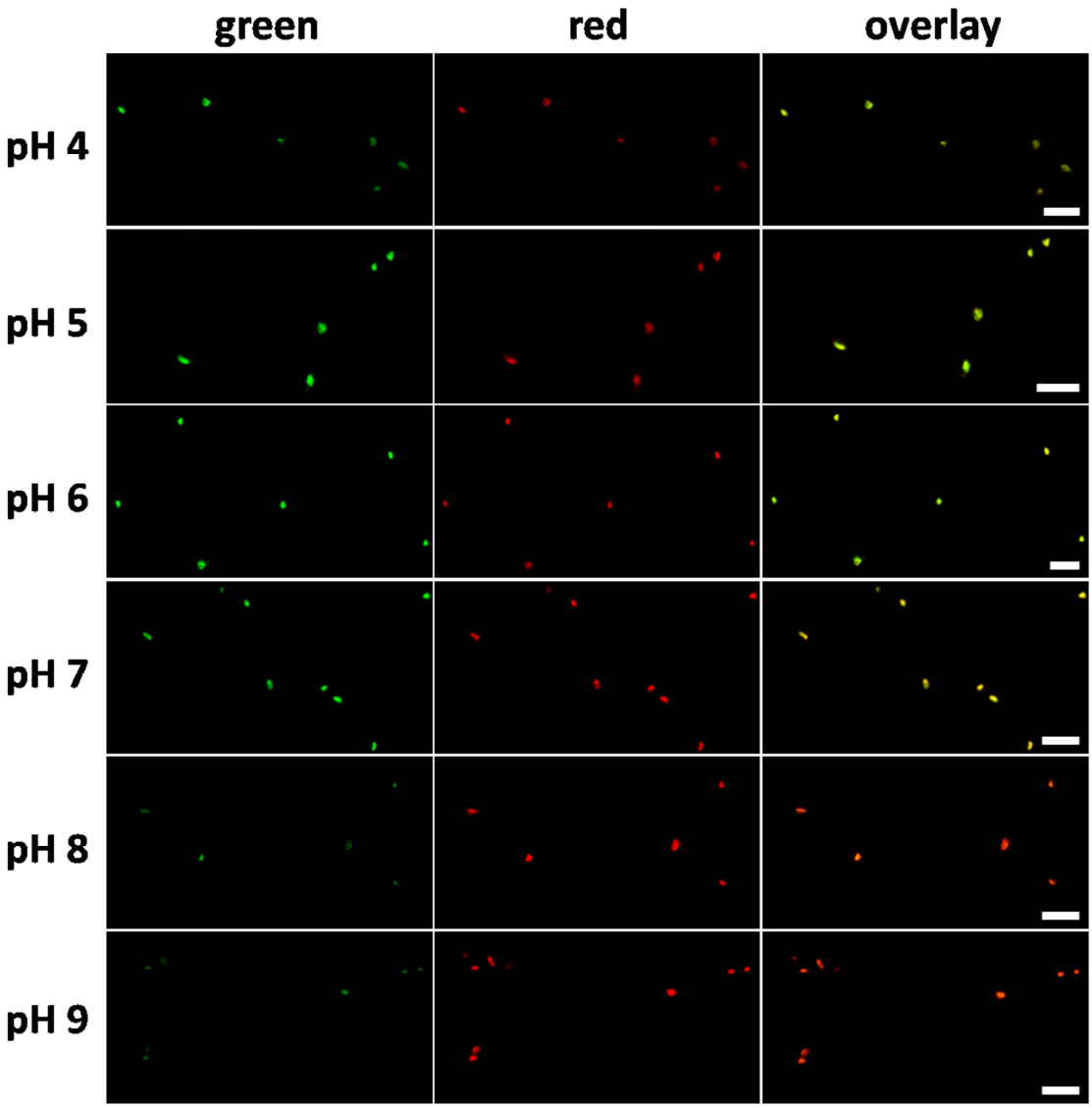

Figure S4. Fiber fluorescence micrographs. Average fiber diameter = 300 nm. The individual green (false color, 540-610 nm) and red (620-700 nm) channels together with channel overlay are shown. Scale bars = 20 μm.

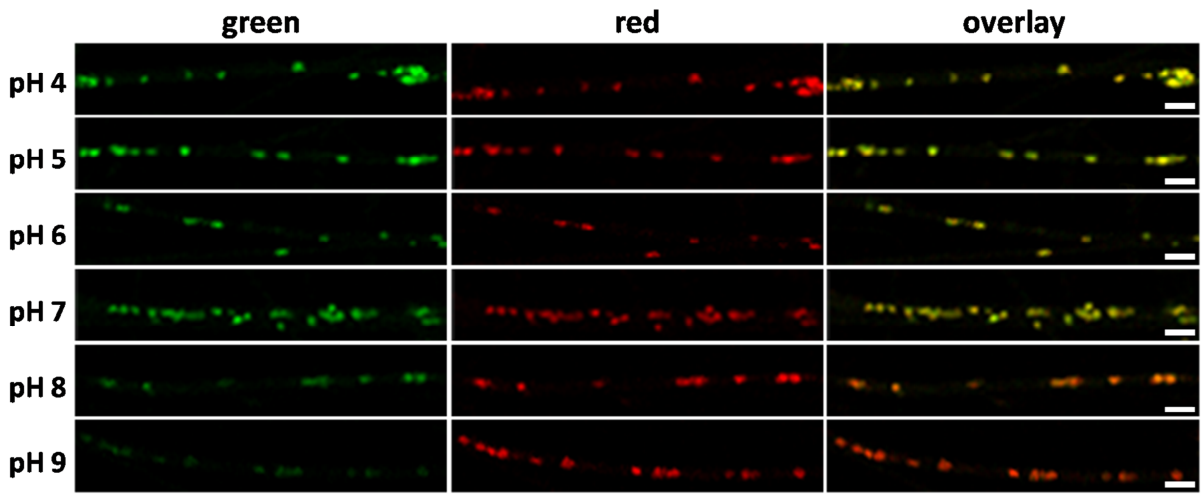

Figure S5. Fiber fluorescence micrographs. Average fiber diameter = 3 μm. The individual green (false color, 540-610 nm) and red (620-700 nm) together with channel overlay are shown. Scale bars = 10 μm.

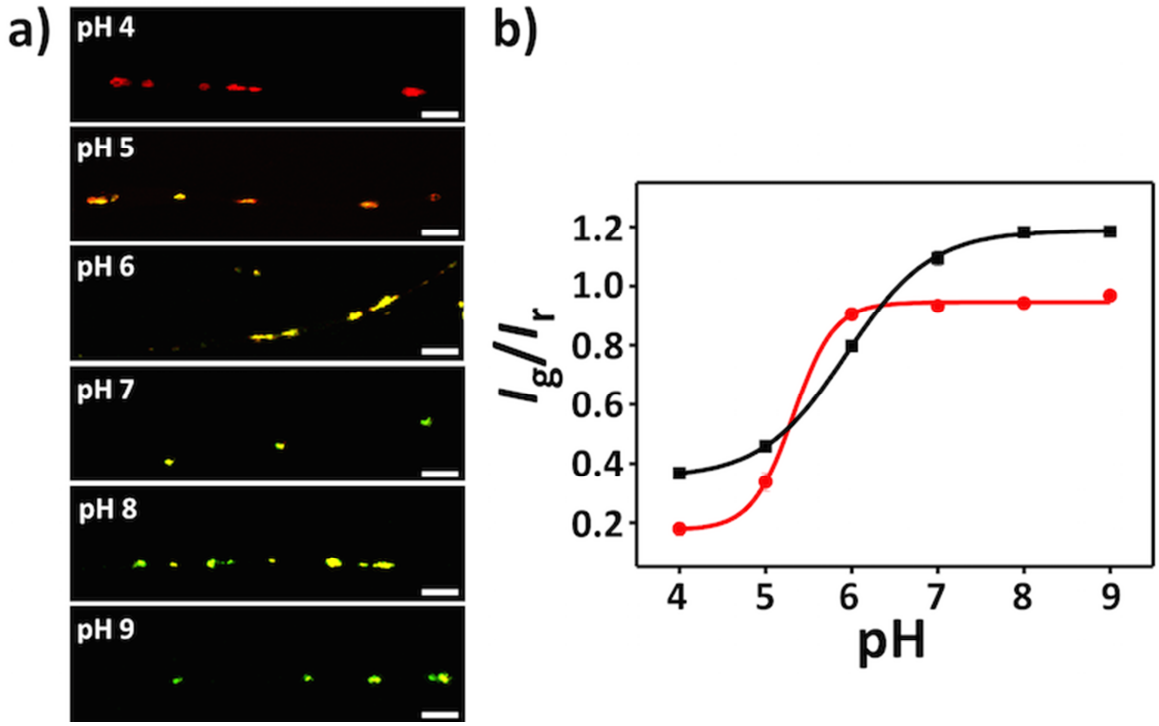

Figure S6. Hybrid fibers embedding capsules loaded with fluorescein 5(6)-isothiocyanate (FITC)- and rhodamine B isothiocyanate (RITC)-dextran conjugates. The pH-sensitive dye, FITC (Mw = 389.38 Da, Sigma), and the reference dye, RITC (Mw = 536.08 Da, Sigma), were covalently linked to the nonfluorescent aminodextran and subsequently co-loaded into the cavities of the capsules as previously described.[1,2] Next, the capsules were embedded within 3 μm-thick PLLA fibers, according to the procedure described in the "Experimental Section" for SNARF-1-dextran conjugate loaded capsules. Finally, the pH-sensitivity of the free capsules and of the fibers was monitored by recording the fluorescent response to various pHs from 4 to 9. (a) CLSM micrographs showing the pH-dependence of fluorescence in pH-adjusted cell medium (λ_{exc} FITC = 488 nm, λ_{exc} RITC = 543 nm). Overlays of the fluorescence channels (green channel: 505−530 nm, red channel: >560 nm) are reported. By increasing the pH of the medium the overall color of the pH-sensing capsules shifts from "red" over "yellow" to "green" as result of the pH dependence of FITC (indicator dye) and the insensitivity of RITC (reference dye). Scale bars: 10 μm. (b) Ratiometric calibration curve of free capsule-based sensors (black squares) and sensors in 3 μm-thick wires (red circles), by fluorescence intensity ratio of green and red channels derived from CLSM micrographs. The green-to-red ratio (false colors) of the fluorescence signal I_g/I_r is here plotted *vs.* the pH of the solution. The data points correspond to the mean ± standard error of mean, calculated over at least 35 capsules. Data were fitted with a sigmoidal function,[2] having points of inflection at pH = 5.94 (black fit) and 5.32 (red fit), respectively.

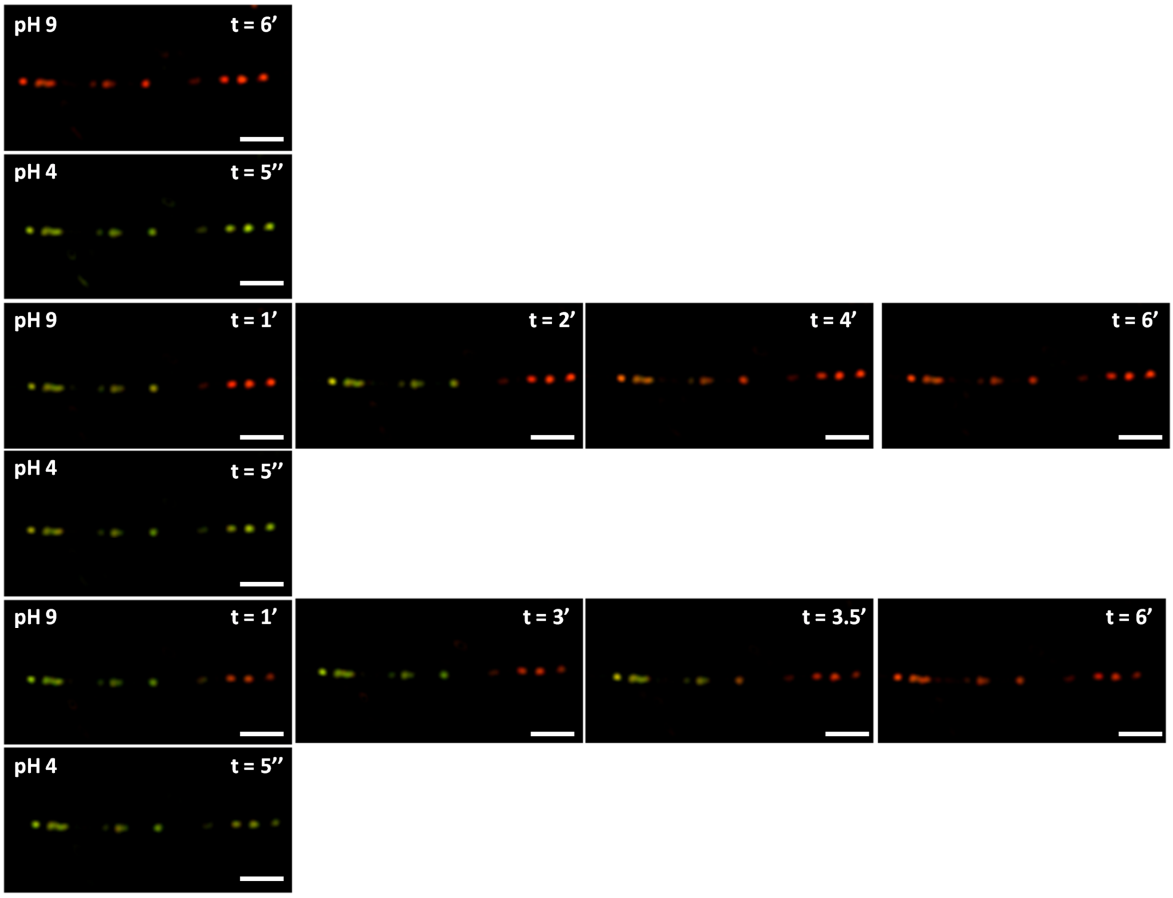

Figure S7. Fluorescence time response to pH variations. The CLSM micrographs show the overlays of the green (false color, 540-610 nm) and red (620-700 nm) channels. The *t* values for each frame indicate the time after the application of a pH change. The analysis show a slower response of 3 μm-thick fibers to basic pHs (9) compared to acidic pHs (4). Average fiber diameter = 3 μm. Scale bars = 20 μm.

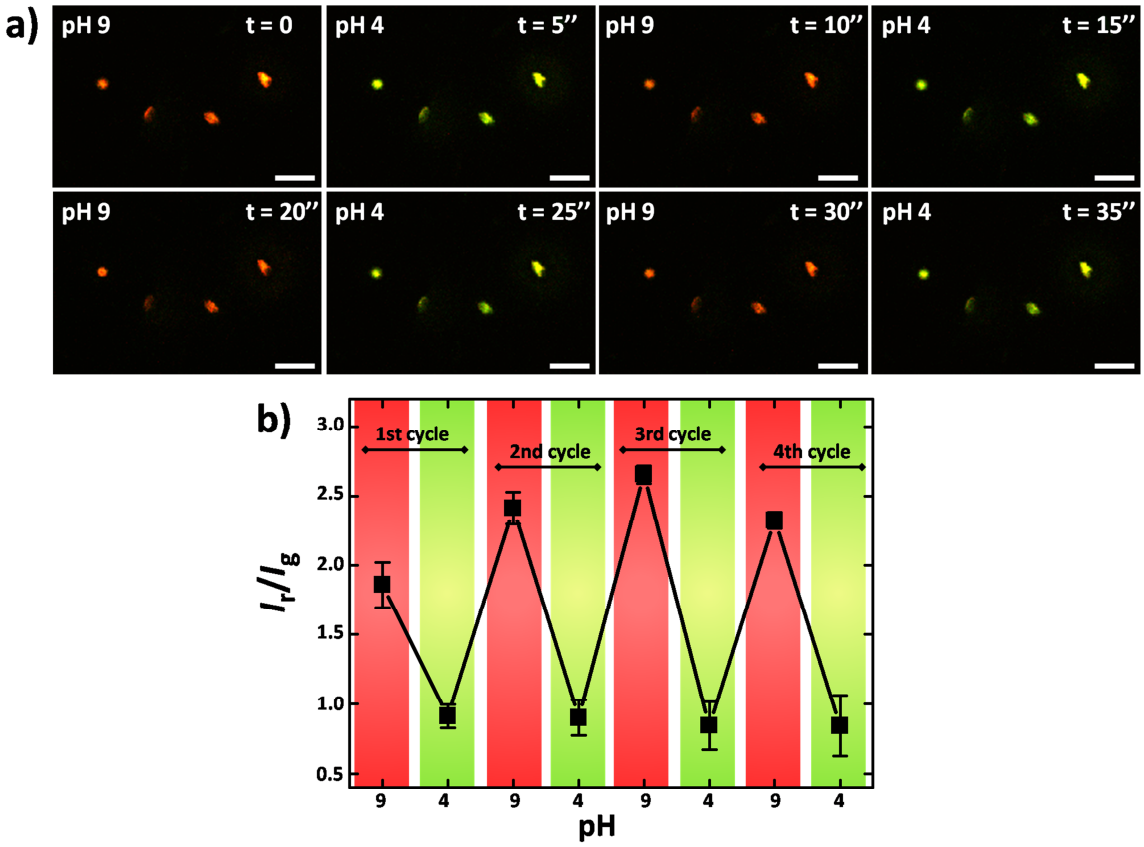

Figure S8. Reversible response of pH-sensing. Fibers were imaged via CLSM after addition of a drop of solution at pH 9. Next, a drop of solution at pH 4 was deposited onto the same region. The cycle was repeated up to 40 times. (a) Overlay of green (false color, 540-610 nm) and red (620-700 nm) channels recorded at each tested pH. The *t* values for each frame indicate the time after the application of a pH change. Average fiber diameter = 300 nm. Scale bars = 10 μm. (b) Red-to-green ratio (false colors) of the fluorescence signal I_r/I_g *vs.* pH.

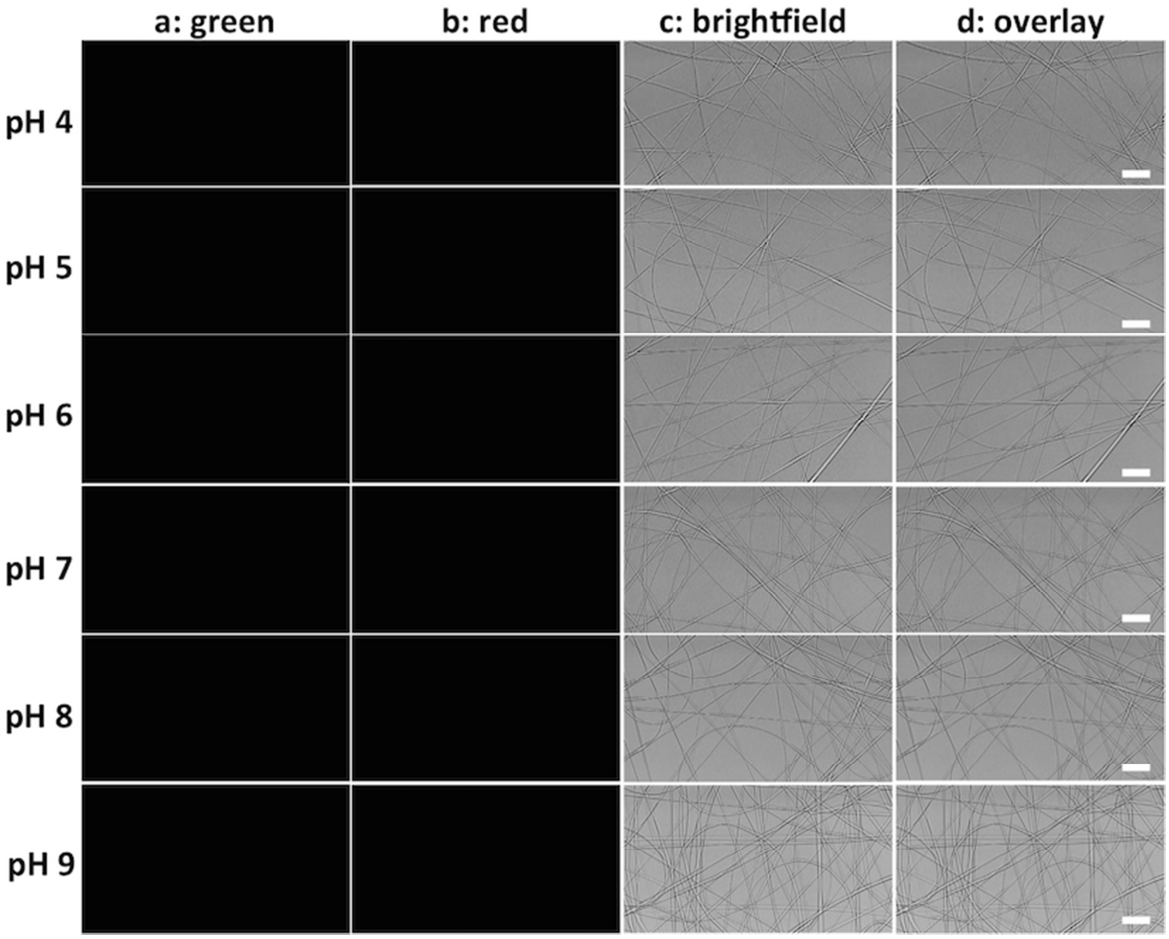

Figure S9. Control experiments. CLSM images of PLLA fibers under different pHs. 20 μL droplets of pH-adjusted buffers (4, 5, 6, 7, 8, and 9) were deposited onto a region of 3 μm-thick fibers (without capsules) for 20 minutes; emission was collected in green channel (λ_{em} = 540–610 nm, a) and red channel (λ_{em} = 620-700 nm, b). λ_{exc} = 514 nm. (c) are bright field images and (d) are corresponding overlay images. Brightness and contrast levels in the bright field channel have been adjusted for better visualizing the fibers. Scale bars: 20 μm. No fluorescence is detected from PLLA fibers under the different pHs.

References

[1] L. L. del Mercato, A. Z. Abbasi, W. J. Parak, *Small* **2011**, 7, 351.

[2] M. De Luca, M. M. Ferraro, R. Hartmann, P. Rivera-Gil, A. Klingl, M. Nazarenus, A. Ramirez, W. J. Parak, C. Bucci, R. Rinaldi, L. L. del Mercato, *ACS Appl. Mater. Interfaces* **2015**, 15, 15052.