Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Surpporting Information
Construction of Spidroin Coacervate Microdroplets and Regulation of Its Morphology

1. Supplementary Table

Name	Sequence (5', 3')	Length (nt)	Mw (g/mol)
DDp	CGCGAATTCGCG-phosphate	12	3726.4

Table S1. Oligonucleotides sequence.

2. Supplementary Figures

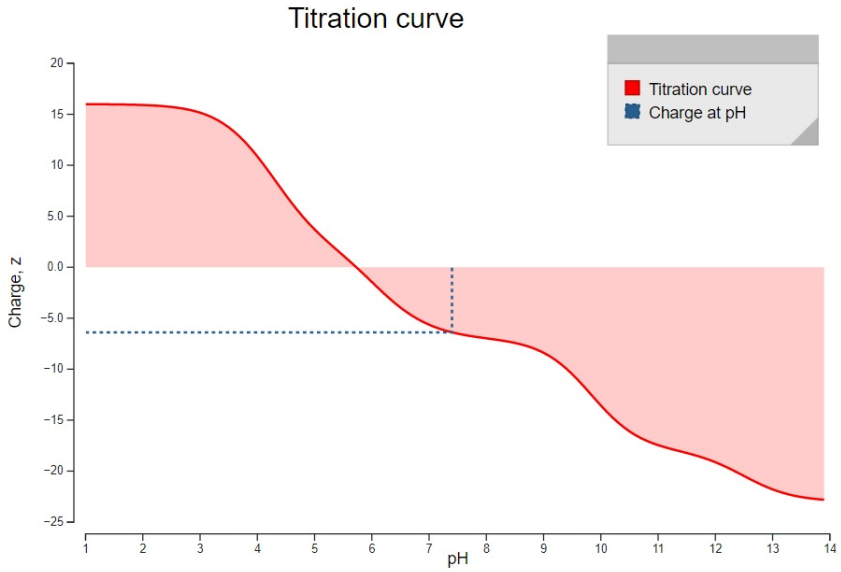

Figure S1. Prediction of isoelectric point (pI) using Prot pi for NT2RepCT.

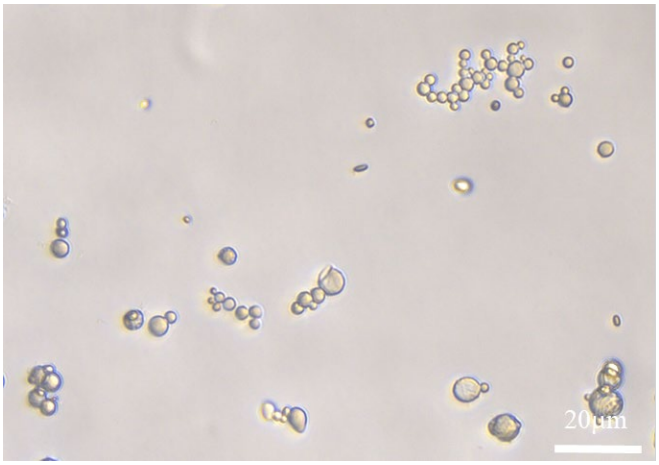

Figure S2. The optical microscopy images of the microdroplets prepared at a concentration of 2 mg/mL by dissolving an appropriate amount of NT2RepCT in PBS buffer (5 mM, pH 6.5). Scale bar, 20 µm.

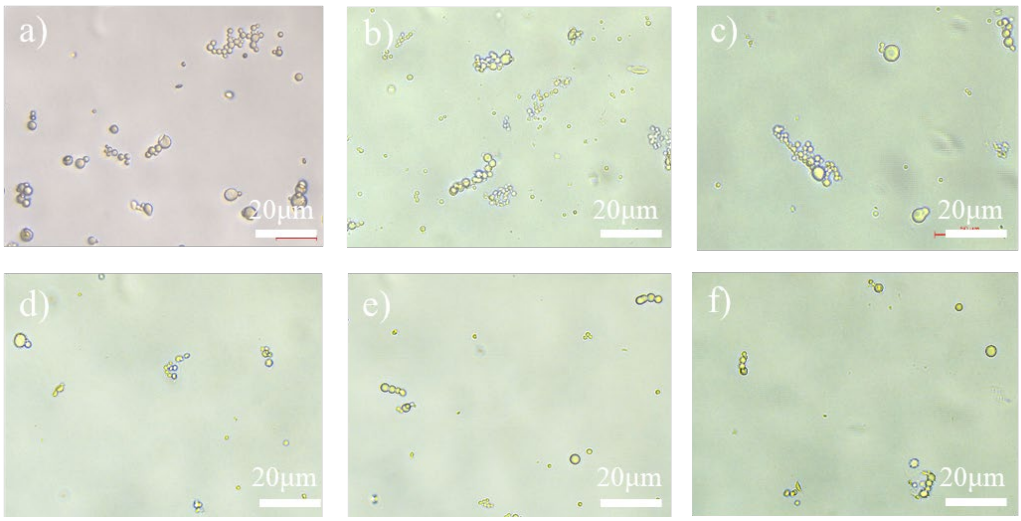

Figure S3. The optical microscopy images of the NT2RepCT coacervate microdroplets prepared at a concentration of 2 mg/mL in the presence of different NaCl concentrations. (a) 0 mM, (b) 200 mM, (c) 400 mM, (d) 600 mM, (e) 800 mM, (f) 3 mM. Scale bar, 20 μm.

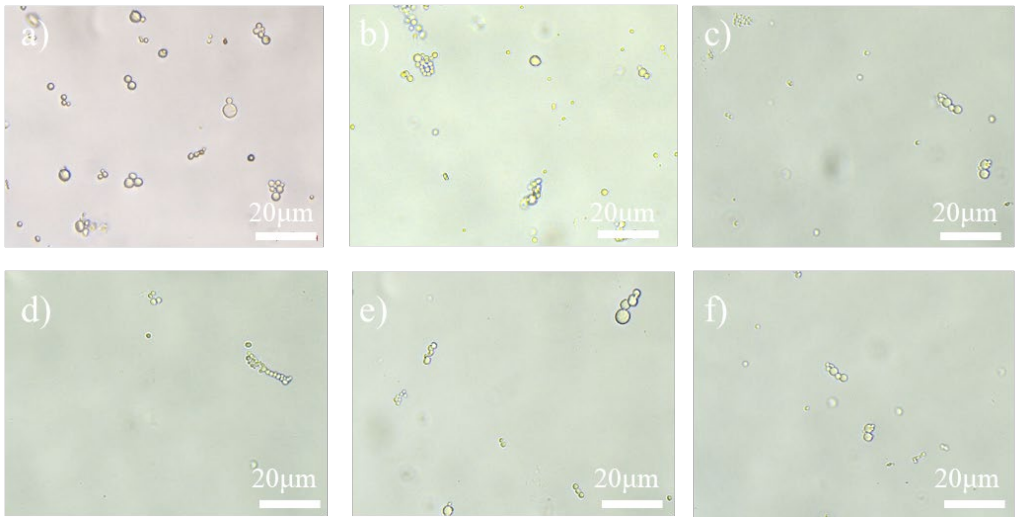

Figure S4. The optical microscopy images of the NT2RepCT coacervate microdroplets prepared at a concentration of 2 mg/mL in the presence of different urea concentrations. (a) 0 M, (b) 1 M, (c) 2 M, (d) 3 M, (e) 4 M, (f) 8 M. Scale bar, 20 μm.

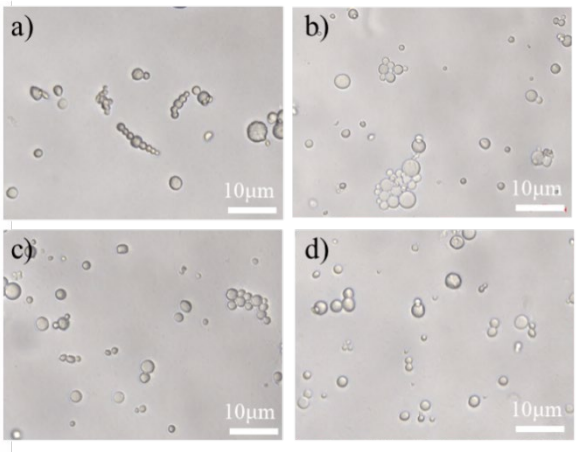

Figure S5. The optical microscopy images of the NT2RepCT coacervate microdroplets prepared at a concentration of 2 mg/mL (a, b) in PBS buffer (5 mM, pH 2.2). (c, d) in PBS buffer (5 mM, pH 10.6). Scale bar, 10 μm.

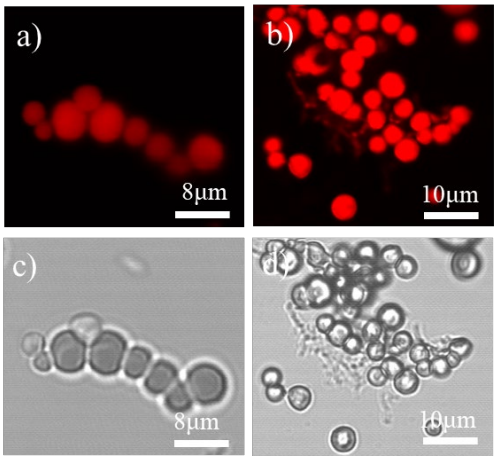

Figure S6. Confocal fluorescence images of RBITC-NT2RepCT prepared at a concentration of 2 mg/mL in PBS buffer (5 mM, pH 6.5). Scale bar (a, c), 8 μm. Scale bar (b, d), 10 μm.

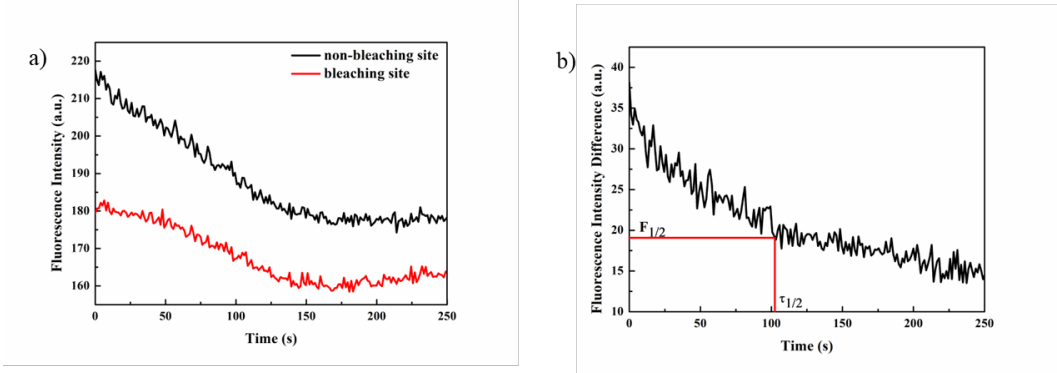

Figure S7. Fluorescence intensity recovery curve of RBITC-NT2RepCT in coacervate phase. After calculation, $\tau_{1/2}$ is 102.37 s, r_n is 0.76 μm, the diffusion coefficient of RBITC-NT2RepCT in coacervate phase (D) was 1.4105×10^{-3} $\mu m^2\times s^{-1}$.

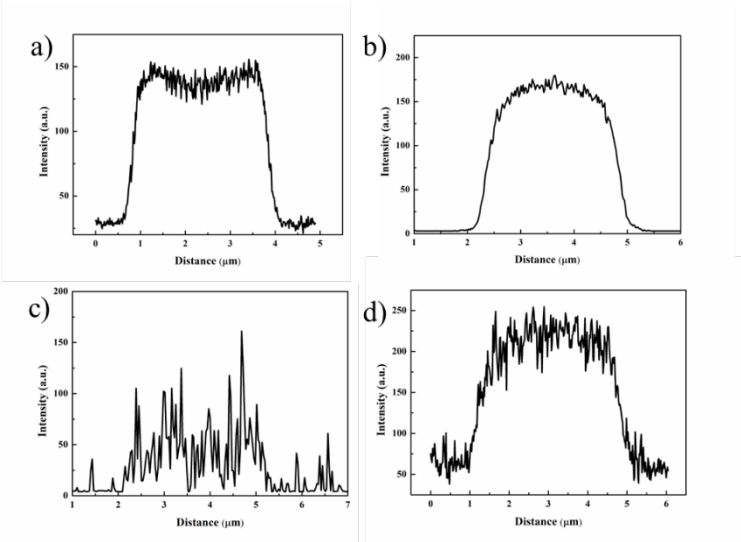

Figure S8. Fluorescence intensity line profiles to explore the sequestration behavior of NT2RepCT coacervate microdroplets in the presence of (a) Rhodamine B, (b) Nile red, (c)MB, (d)Fluorescein Sodium (final concentrations, 0.25 mM.)

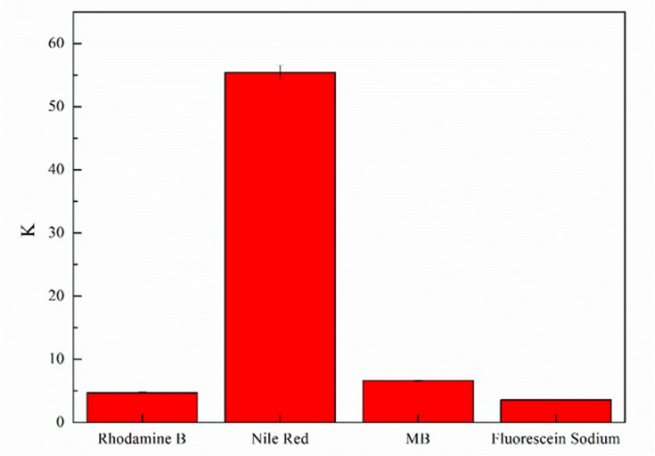

Figure S9. The partition coefficient K of Rhodamine B, Nile red, MB, Fluorescein Sodium in **Figure S8**. $K_{\text{Rhodamine B}}$ was 4.7132, $K_{\text{Nile Red}}$ was 55.4127, K_{MB} was 6.6362 and $K_{\text{Fluorescein Sodium}}$ was 3.5557. Errors bars represent standard deviation of the partition coefficient K (calculated from different coacervate microdroplets in each image).

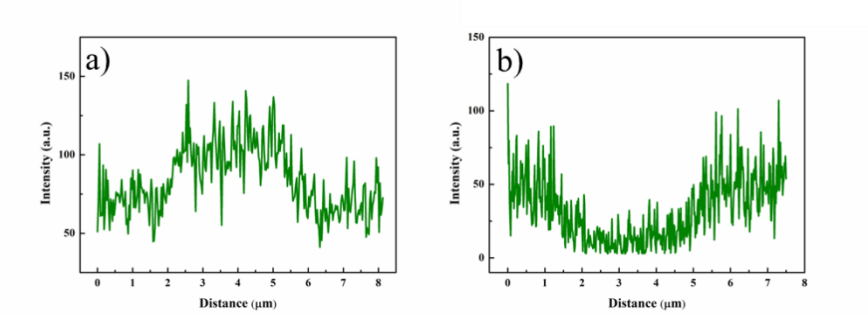

Figure S10. Fluorescence intensity line profiles to explore the sequestration behavior

of NT2RepCT coacervate microdroplets in the presence of FITC-dextran, (a) Mw=4 kDa, (b) Mw=10 kDa. $K_{FITC\text{-}Dextran\ (4\ kDa)}$ was 1.4937, $K_{FITC\text{-}Dextran\ (10\ kDa)}$ was 0.2503.

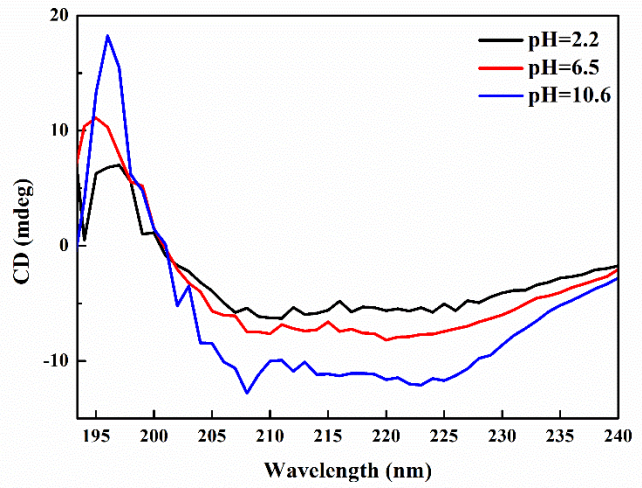

Figure S11. CD spectra of NT2RepCT in PBS buffers (5 mM) of various pH conditions. After calculation via BESTSEL, α-helical and β-sheet accounted for 59.1 % and 37.2 % at pH 2.2, while α-helical and β-sheet accounted for 77.6 % and 22.5 % at pH 6.5, α-helical and β-sheet accounted for 68.8 % and 31.2 % at pH 10.6.

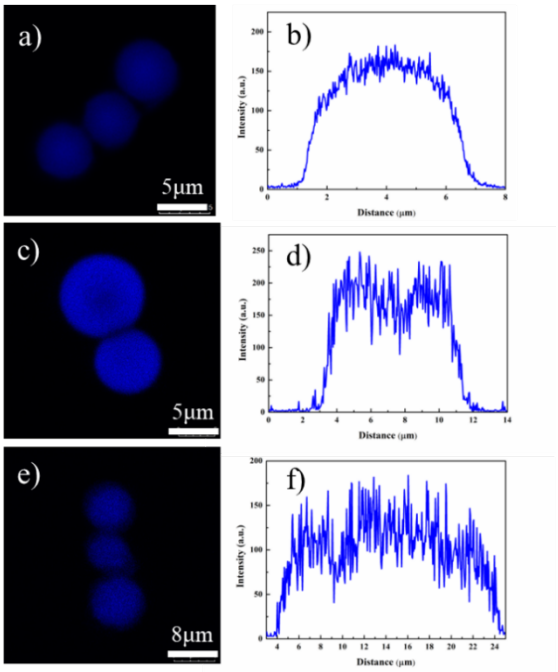

Figure S12. (a) Confocal fluorescence images of thiosemicarbazone T in coacervate microdroplets prepared in PBS buffer (5 mM, pH 6.5). Scale bar, 5 μm. (b) Fluorescence intensity line profiles in **Figure S12** (a), K_{ThT} (pH 6.5) was 31.1468. (c) Confocal fluorescence images of thiosemicarbazone T in coacervate microdroplets prepared in PBS buffer (5 mM, pH 2.2). Scale bar, 5 μm. (d) Fluorescence intensity line profiles in **Figure S12** (c), K_{ThT} (pH 2.2) was 42.2877. (e) Confocal fluorescence images of thiosemicarbazone T in coacervate microdroplets prepared in PBS buffer (5

mM, pH 10.6). Scale bar, 8 μm. (f) Fluorescence intensity line profiles in **Figure S12** (e), K_{ThT} (pH 10.6) was 35.4349.

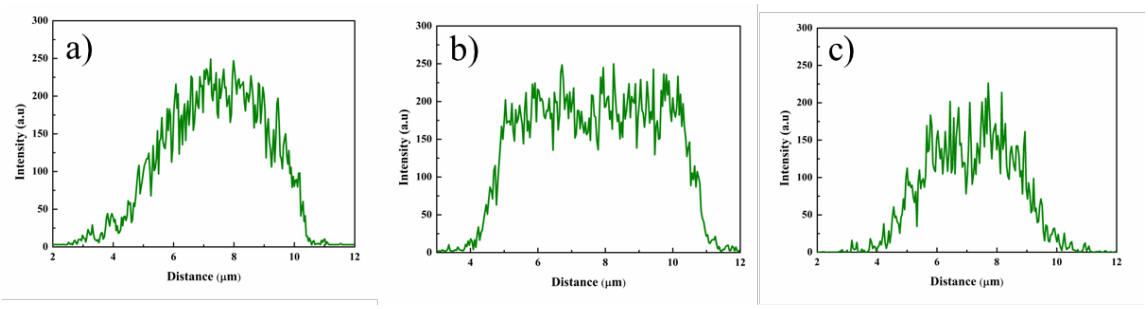

Figure S13. Fluorescence intensity line profiles in **Figure 4c.** (a) 0 h, (b) 24 h, (c) 48h.

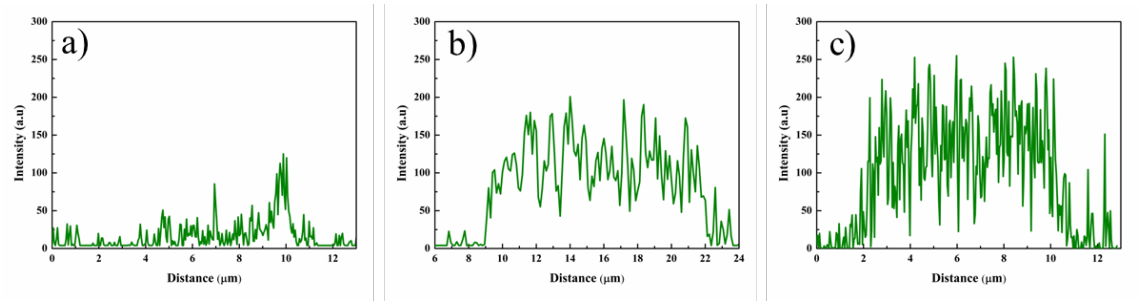

Figure S14. Fluorescence intensity line profiles in **Figure 4e.** (a) 0 h, (b) 24 h, (c) 48h.

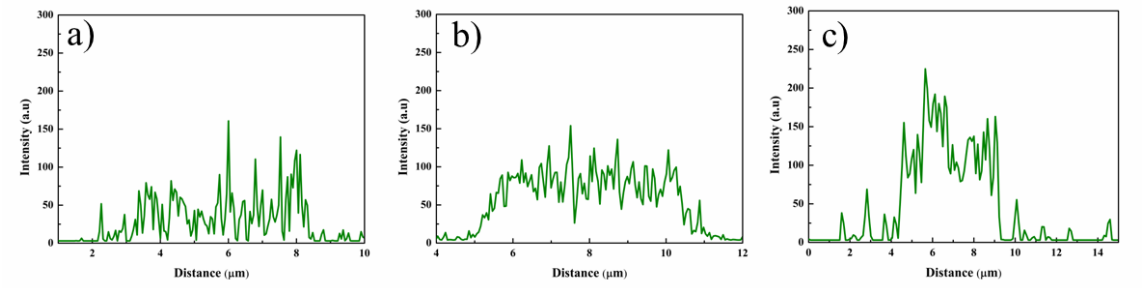

Figure S15. Fluorescence intensity line profiles in **Figure 4g.** (a) 0 h, (b) 24 h, (c) 48h.