

Supplementary Material (ESI) for Journal of Materials Chemistry
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Electronic Supplementary Information (ESI)

Tunable dual-emitting shell-crosslinked nano-objects as single-component ratiometric pH-sensing materials

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Preparation of SCR-Bs: To a 50-mL round bottom flask equipped with a magnetic stir bar was added a solution of **1** in nanopure H₂O (22 mL or 21 mL, 57 μmol or 54 μmol of carboxylic acid residues). To this solution, was added a solution of **B** (0.30 mg, 0.45 μmol (0.79 mol% relative to the acrylic acid residues) for 2% crosslinking extent; 1.5 mg, 2.3 μmol (3.9 mol% relative to the acrylic acid residues) for 7% crosslinking extent; or 3.0 mg, 4.4 μmol (7.9 mol% relative to the acrylic acid residues) for 12% crosslinking extent). The reaction mixture was allowed to stir at rt for 2 h. To this solution was added, dropwise *via* a syringe pump over 1 h, a solution of 1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDCI): 0.34 mg, 1.1 μmol (stoichiometric) for 2% crosslinking extent; 1.7 mg, 5.7 μmol (stoichiometric) for 7% crosslinking extent; 3.4 mg, 11 μmol (stoichiometric) for 12% crosslinking extent); 0.52 mg, 1.8 μmol (2 molar excess) for 2% crosslinking extent; 2.6 mg, 8.8 μmol (2 molar excess) for 7% crosslinking extent or 5.2 mg, 18 μmol (2 molar excess) for 10% crosslinking extent and the reaction mixture was further stirred at rt for 16 h. Finally, the reaction mixture was transferred to pre-soaked dialysis tubing (MWCO *ca.* 3,500 Da) and dialyzed against 5 mM PBS (5 mM NaCl, pH 7.4) for a day then nanopure water for another day to remove the non-attached crosslinker, excess small molecule starting materials and by-products, and afford aqueous solutions of shell-crosslinked cylinder, **SCR-B2%**, **SCR-B7%**, **SCR-B12%**, **SCR-B2%**, **SCR-B7%** or **SCR-B10%** (final polymer concentration: 0.30 mg/mL, 0.29 mg/mL or 0.28 mg/mL for stoichiometric addition of EDCI and 0.23 mg/mL, 0.23 mg/mL or 0.23 mg/mL for 2 molar excess amount of EDCI, respectively—where in each case, the % crosslinking was determined by UV-vis spectroscopic measurement of the amount of crosslinker remaining after purification). SCR solutions for UV-vis, and fluorescence studies were further partitioned into four vials each containing 5 mM PBS (with 5 mM NaCl) at pH values of 4.6, 6.4, 7.4 and 8.4. SCRs measured 23 ± 2 nm in width and 100 nm to a micron length, by TEM.

Preparation of SCR-Cs: To a 50-mL round bottom flask equipped with a magnetic stir bar was added a solution of **1** in nanopure H₂O (28 mL or 21 mL, 72 μmol or 54 μmol of carboxylic acid residues). To this solution, was added a solution of **C** (0.34 mg, 0.56 μmol (0.79 mol% relative to the acrylic acid residues) for 2% crosslinking extent; 1.7 mg, 2.8 μmol (3.9 mol% relative to the acrylic acid residues) for 7% crosslinking extent; or 3.4 mg, 5.6 μmol (7.9 mol% relative to the acrylic acid residues) for 14% crosslinking extent). The reaction mixture was allowed to stir at rt for 2 h. To this solution was added, dropwise *via* a syringe pump over 1 h, a solution of 1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDCI): 0.42 mg, 1.4 μmol (stoichiometric) for 2% crosslinking extent; 2.1 mg, 7.2 μmol (stoichiometric) for 7% crosslinking extent; 4.3 mg, 14 μmol (stoichiometric) for 14% crosslinking extent; 0.52 mg, 1.8 μmol (2 molar excess) for 2% crosslinking extent; 2.6 mg, 8.8 μmol (2 molar excess)

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for 6% crosslinking extent or 5.2 mg, 17 μmol (2 molar excess) for 3% crosslinking extent) and the reaction mixture was further stirred at rt for 16 h. Finally, the reaction mixture was transferred to pre-soaked dialysis tubing (MWCO *ca.* 3,500 Da) and dialyzed against 5 mM PBS (5 mM NaCl, pH 7.4) for a day then nanopure water for another day to remove the non-attached crosslinker, excess small molecule starting materials and by-products, and afford aqueous solutions of shell-crosslinked cylinder, **SCR-C2%**, **SCR-C7%**, **SCR-C14%**, **SCR-C2%**, **SCR-C6%** or **SCR-C3%** (final polymer concentration: 0.29 mg/mL, 0.28 mg/mL or 0.27 mg/mL for stoichiometric addition of EDCI and 0.23 mg/mL, 0.23 mg/mL or 0.22 mg/mL for 2 molar excess amount of EDCI, respectively—where in each case, the % crosslinking was determined by UV-vis spectroscopic measurement of the amount of crosslinker remaining after purification). SCR solutions for UV-vis, and fluorescence studies were further partitioned into four vials each containing 5 mM PBS (with 5 mM NaCl) at pH values of 4.6, 6.4, 7.4 and 8.4. SCRs measured 23 ± 2 nm in width and 100 nm to a micron length, by TEM.

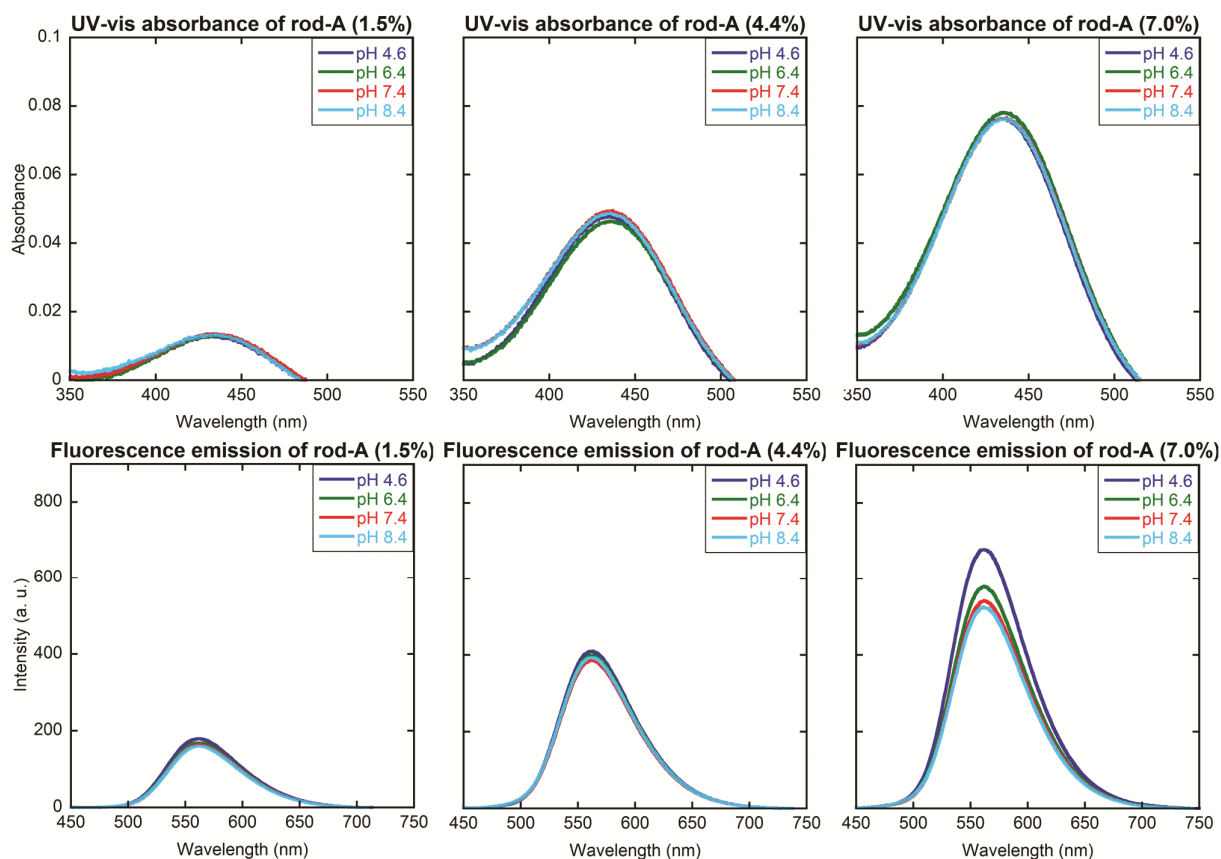


Figure S1. UV-vis absorbance and fluorescence emission spectra of physical mixture of rod-shaped micelles and chromophoric crosslinker A at 1.5, 4.4 or 7.0% loading, based upon the molar percentage of aliphatic amine of the crosslinker to the carboxylic acid groups of the block copolymer assemblies.

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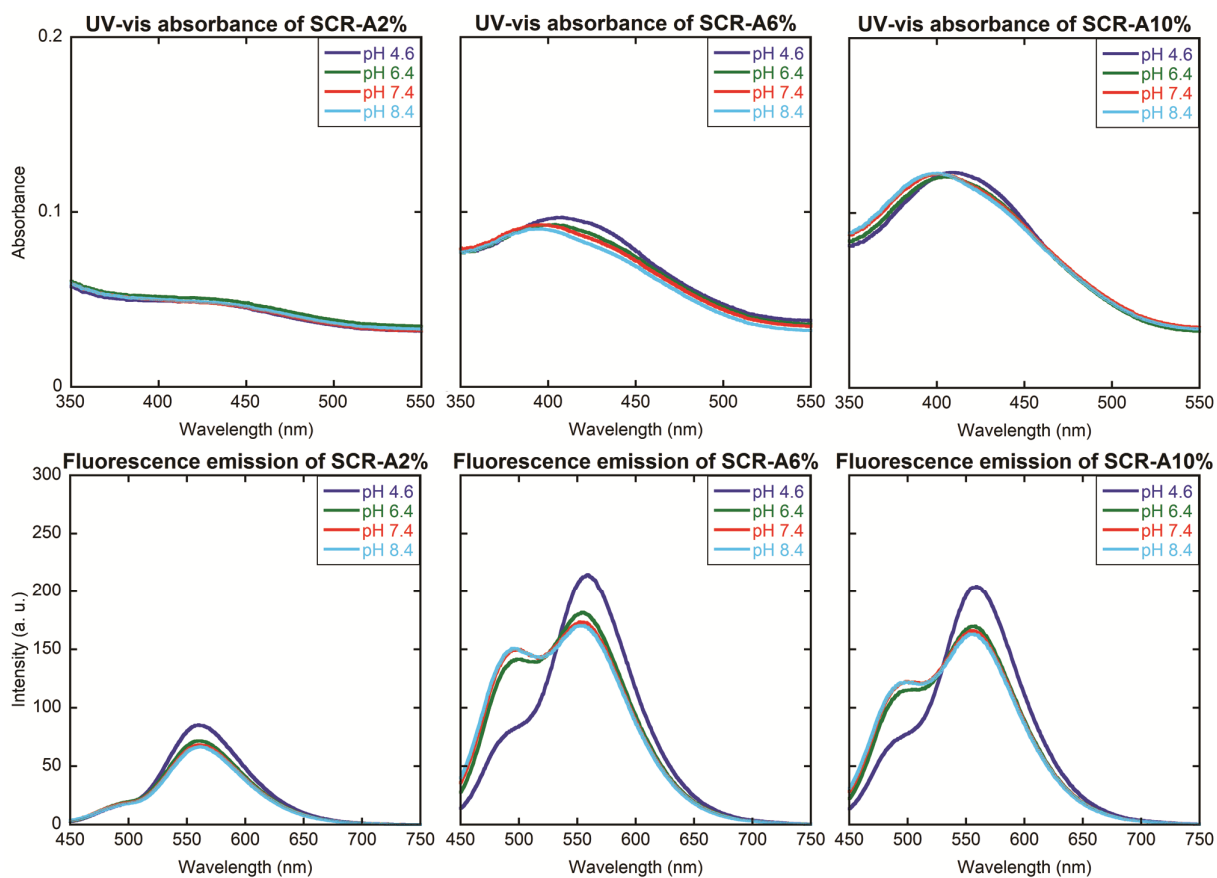


Figure S2. UV-vis absorbance and fluorescence emission spectra of SCR-A at 2%, 6% or 10% crosslinking density with the addition of a stoichiometric amount of EDCl, relative to the aliphatic amines of the crosslinker.

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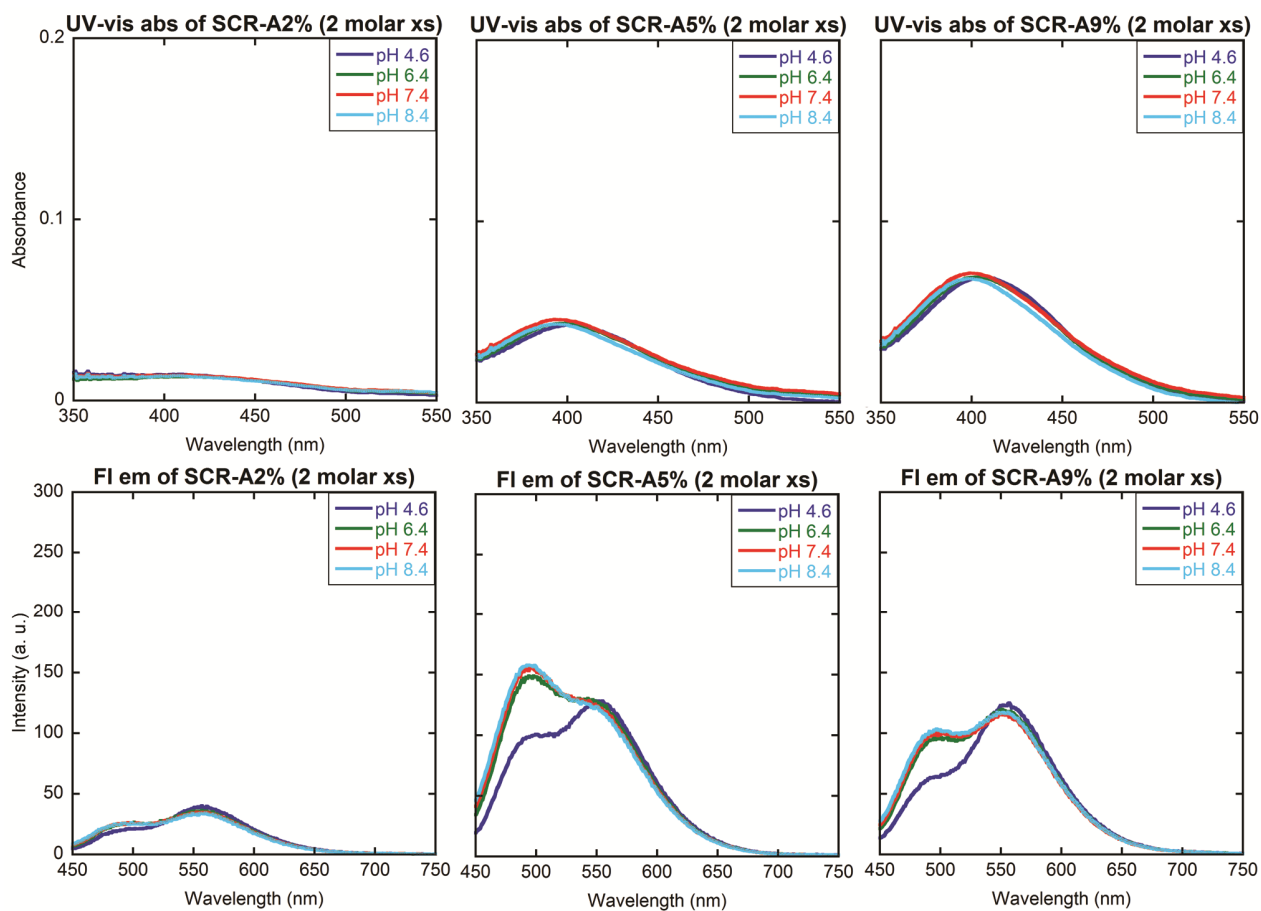


Figure S3. UV-vis absorbance and fluorescence emission spectra of SCR-A at 2%, 5% or 9% crosslinking density with the addition of a 2 molar excess amount of EDCl, relative to the aliphatic amines of the crosslinker.

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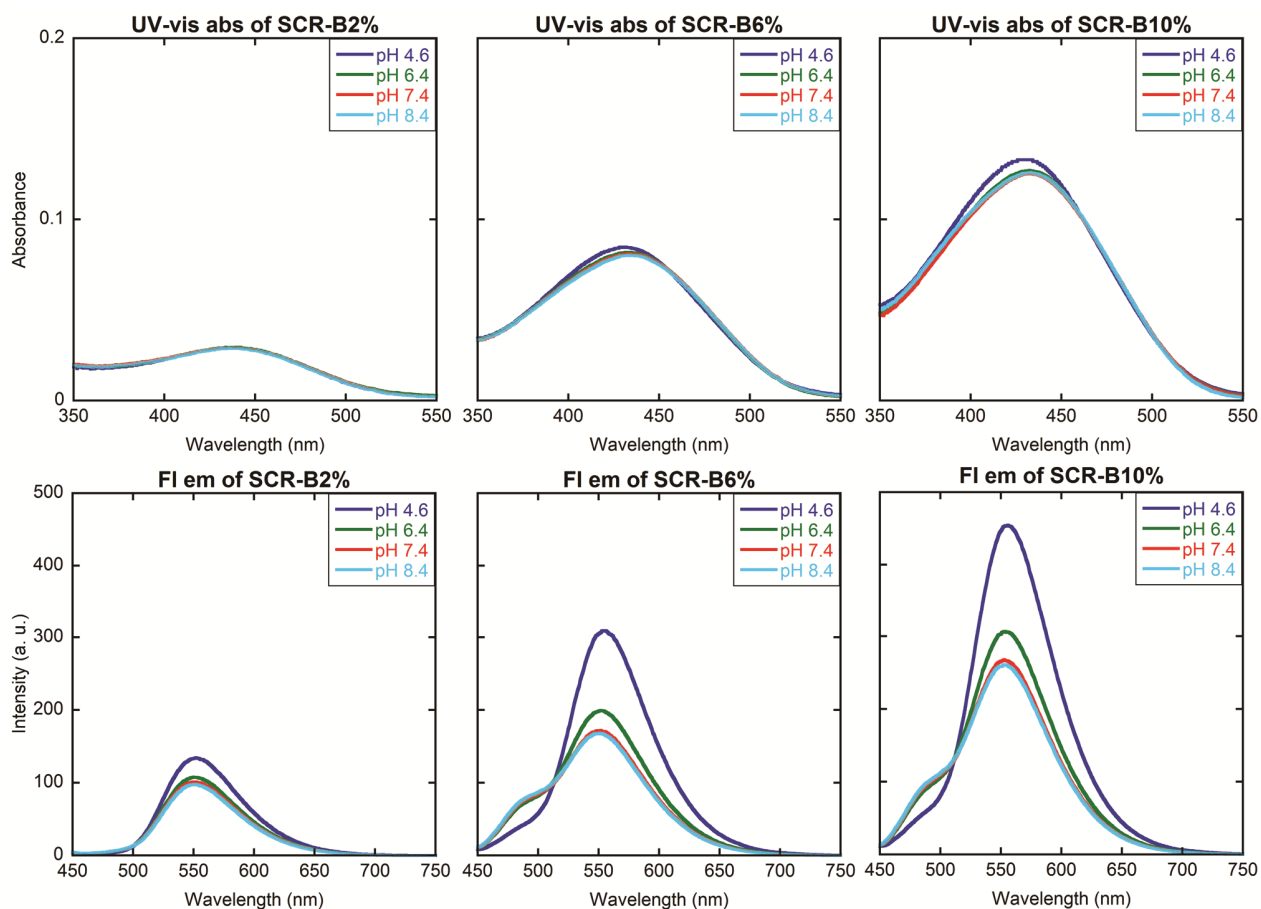


Figure S4. UV-vis absorbance and fluorescence emission spectra of SCR-B at 2%, 6% or 10% crosslinking density with the addition of a stoichiometric amount of EDCl, relative to the aliphatic amines of the crosslinker.

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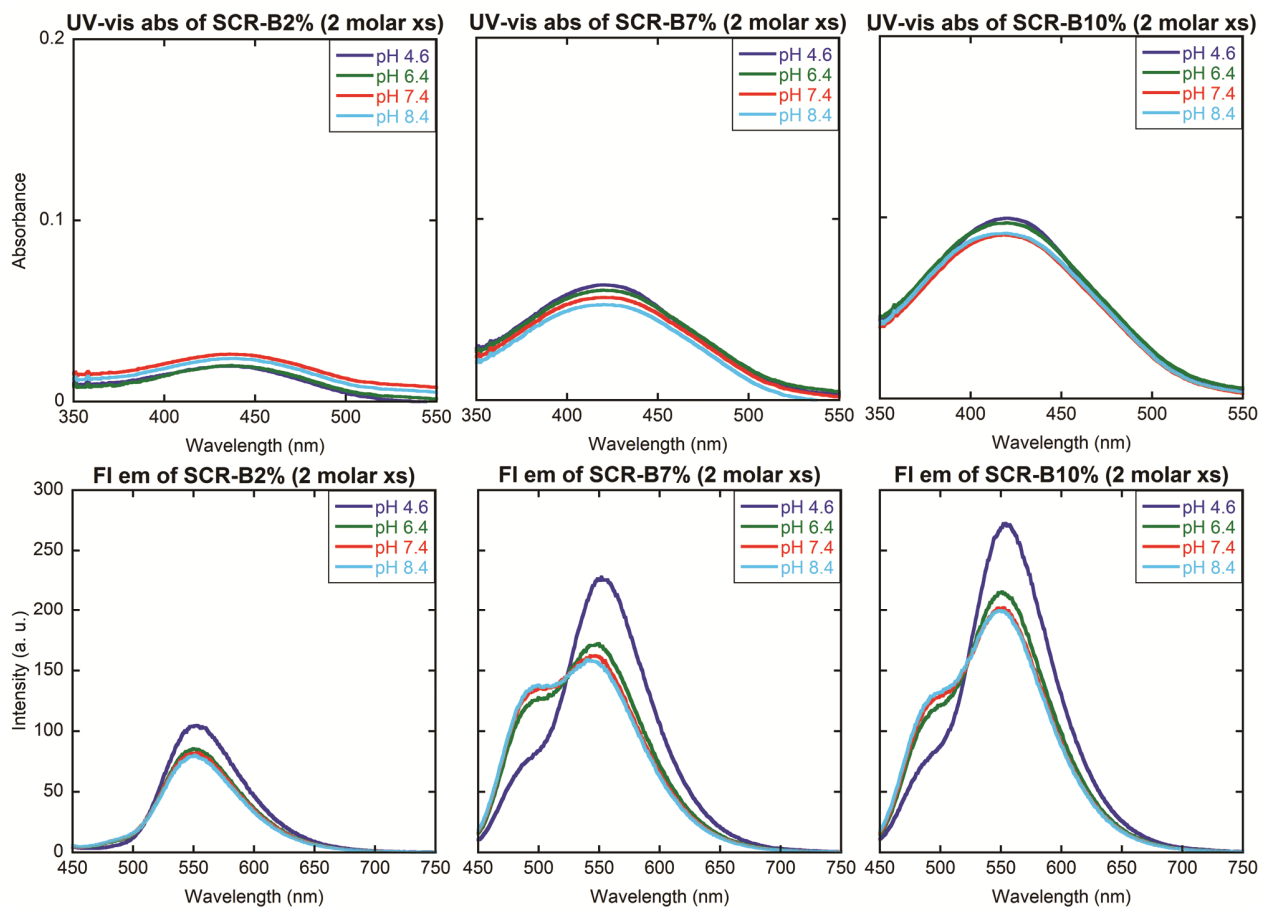


Figure S5. UV-vis absorbance and fluorescence emission spectra of SCR-B at 2%, 7% or 10% crosslinking density with the addition of a 2 molar excess amount of EDCI, relative to the aliphatic amines of the crosslinker.

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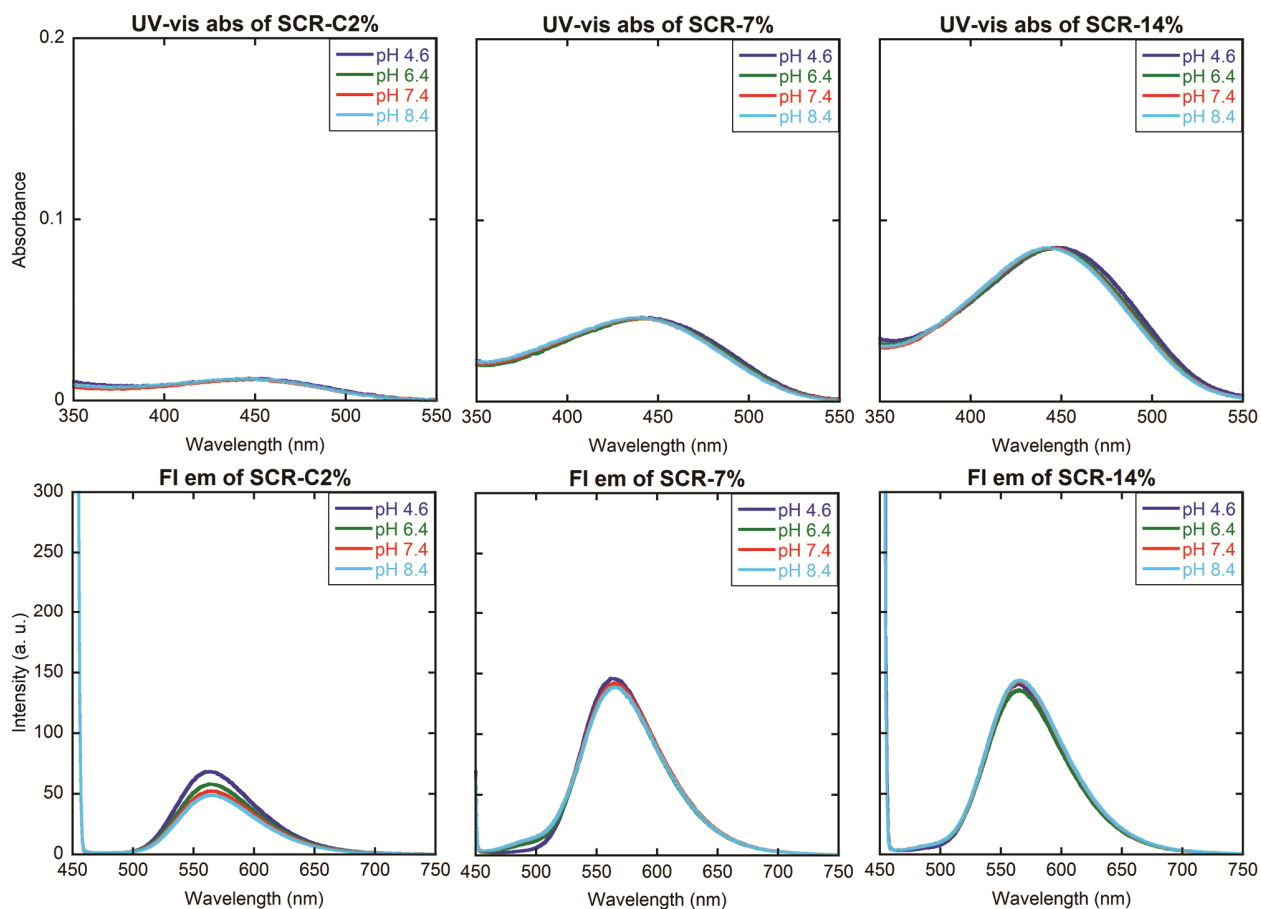


Figure S6. UV-vis absorbance and fluorescence emission spectra of SCR-C at 2%, 7% or 14% crosslinking density with the addition of a stoichiometric amount of EDCI, relative to the aliphatic amines of the crosslinker.

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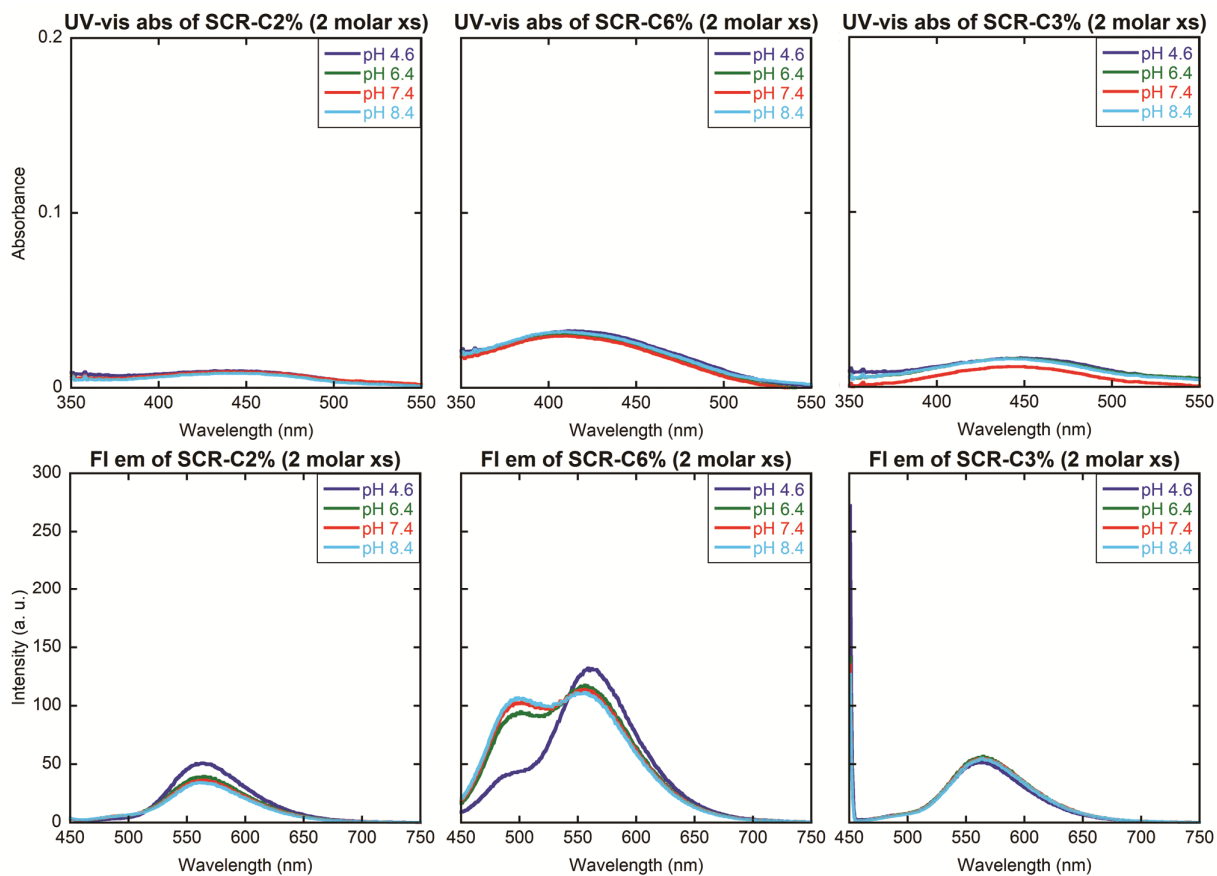


Figure S7. UV-vis absorbance and fluorescence emission spectra of SCR-C at 2%, 6% or 3% crosslinking density with the addition of a 2 molar excess amount of EDCI, relative to the aliphatic amines of the crosslinker.

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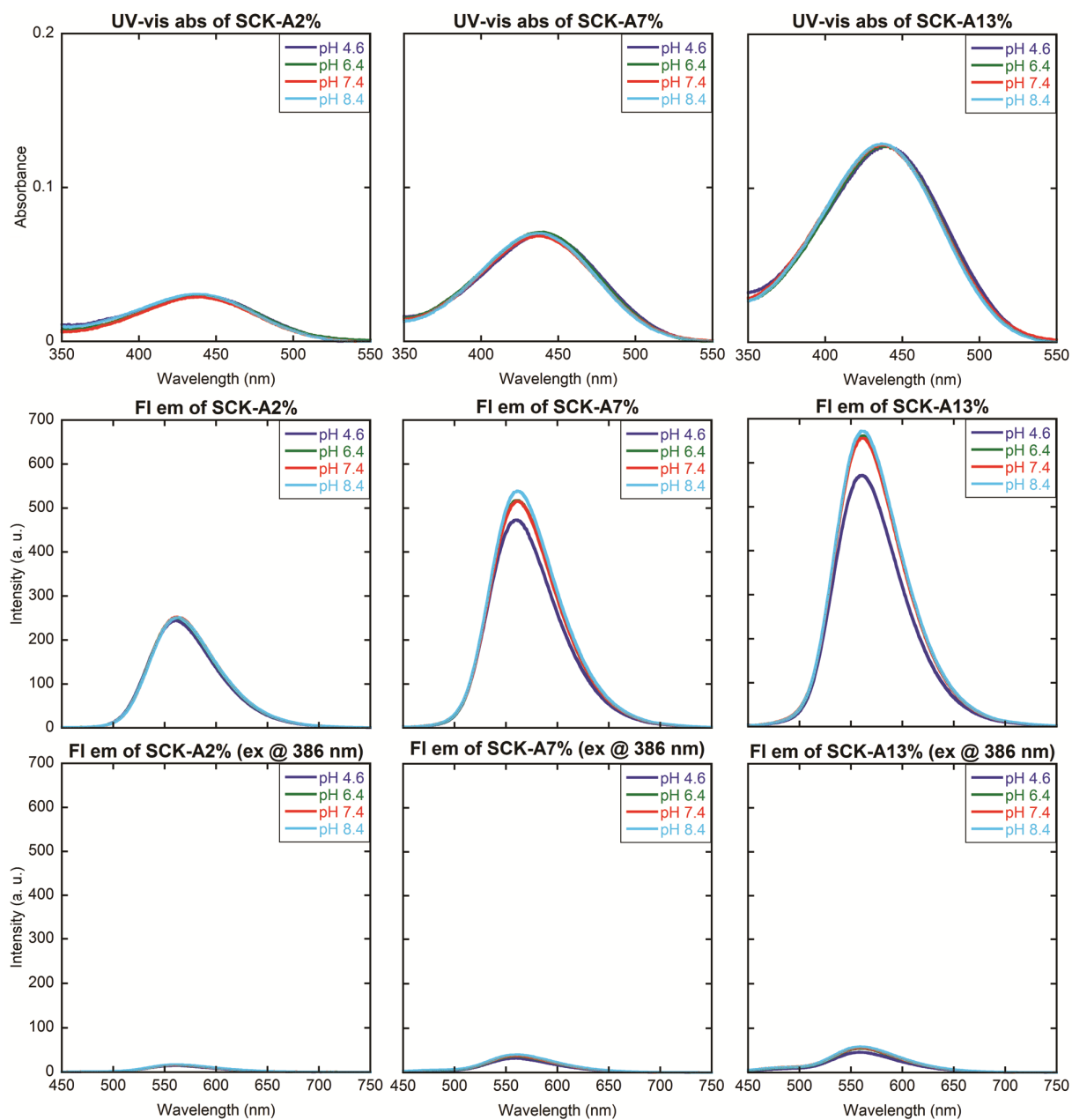


Figure S8. UV-vis absorbance and fluorescence emission spectra of SCK-A at 2%, 7% or 13% crosslinking density with the addition of a stoichiometric amount of EDCI, relative to the aliphatic amines of the crosslinker.

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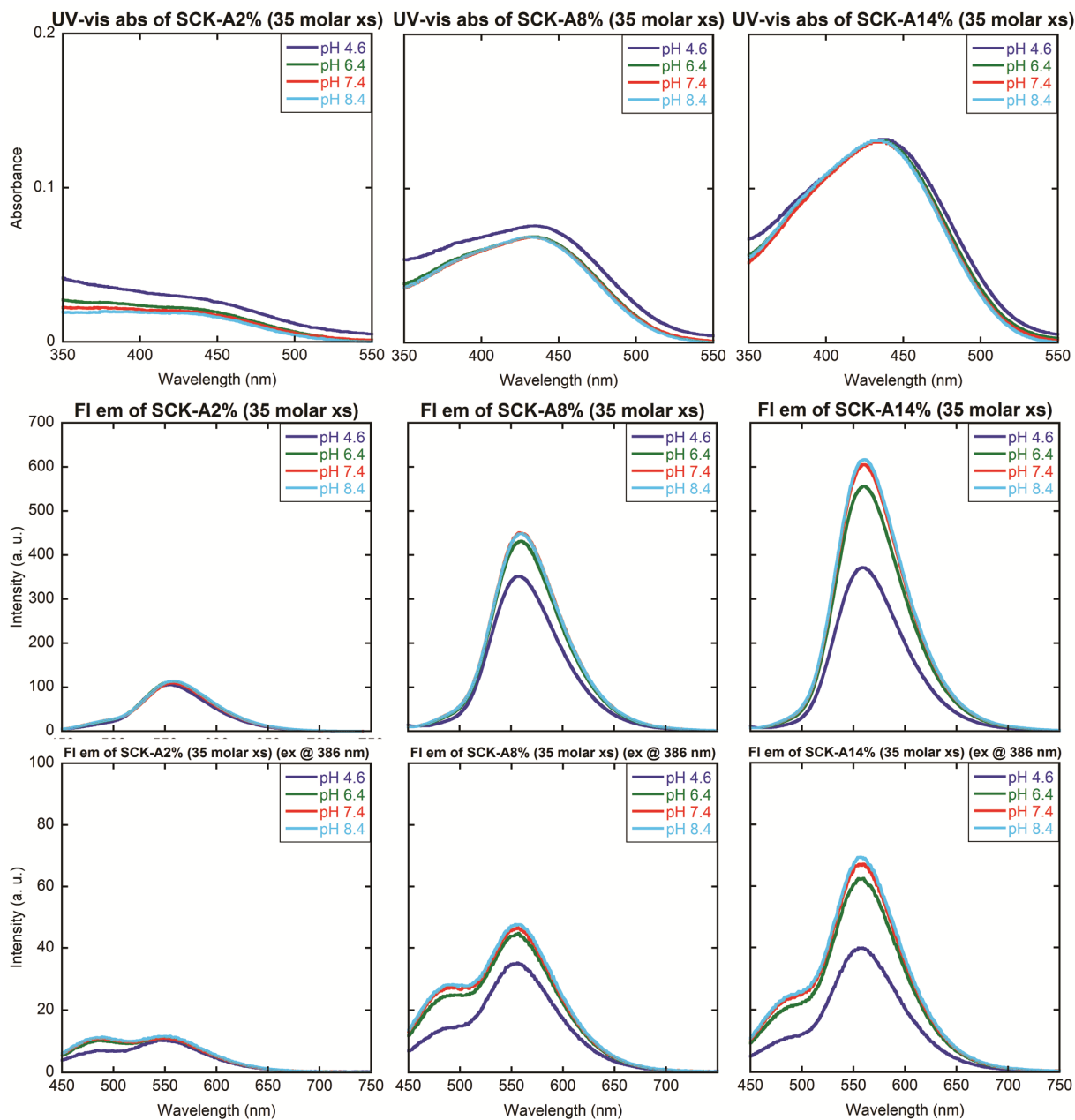


Figure S9. UV-vis absorbance and fluorescence emission spectra of SCK-A at 2%, 8% or 14% crosslinking density with the addition of a 35 molar excess amount of EDCl, relative to the aliphatic amines of the crosslinker.

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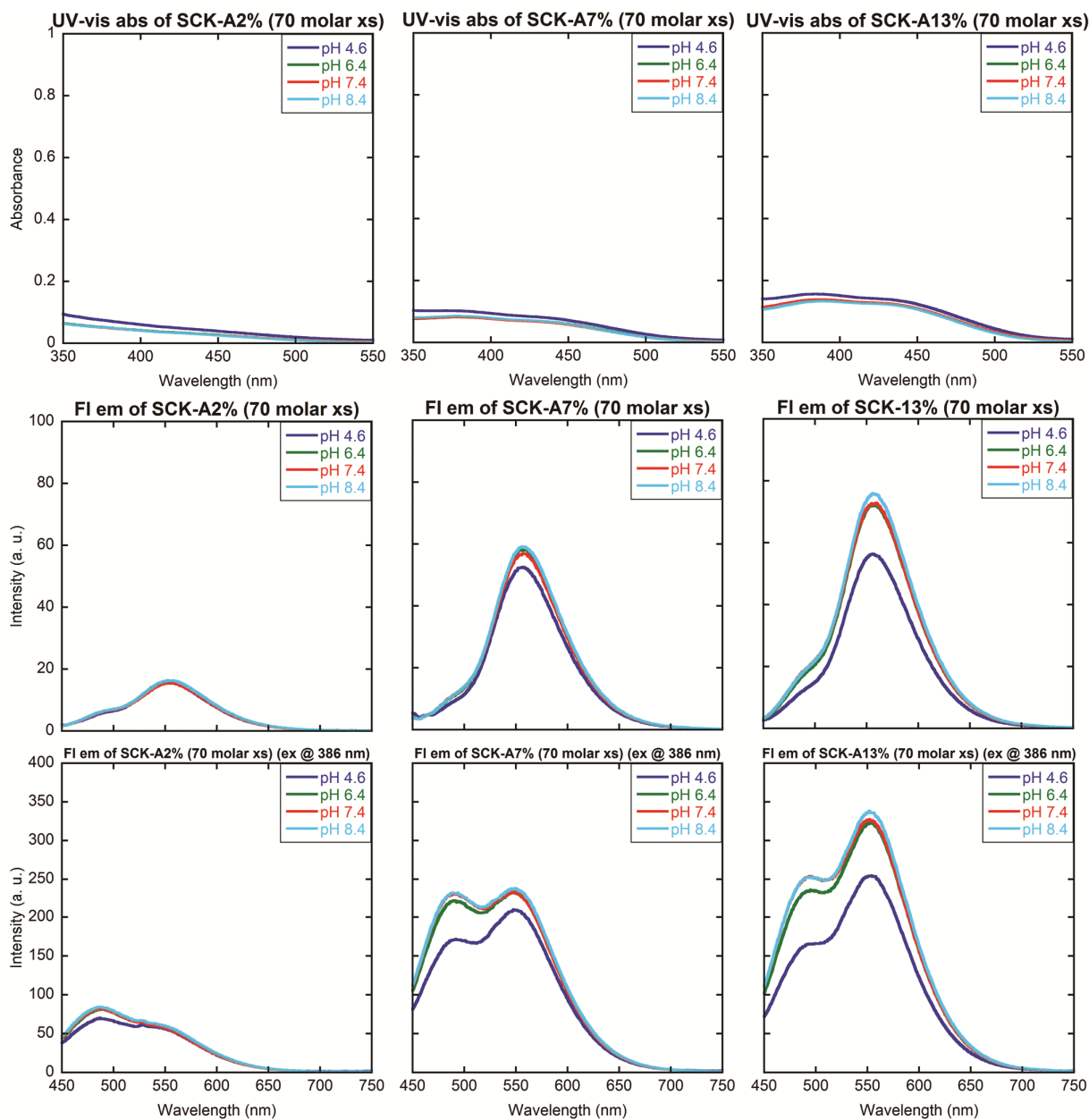


Figure S10. UV-vis absorbance and fluorescence emission spectra of SCK-A at 2%, 7% or 13% crosslinking density with the addition of a 75 molar excess amount of EDCI, relative to the aliphatic amines of the crosslinker.

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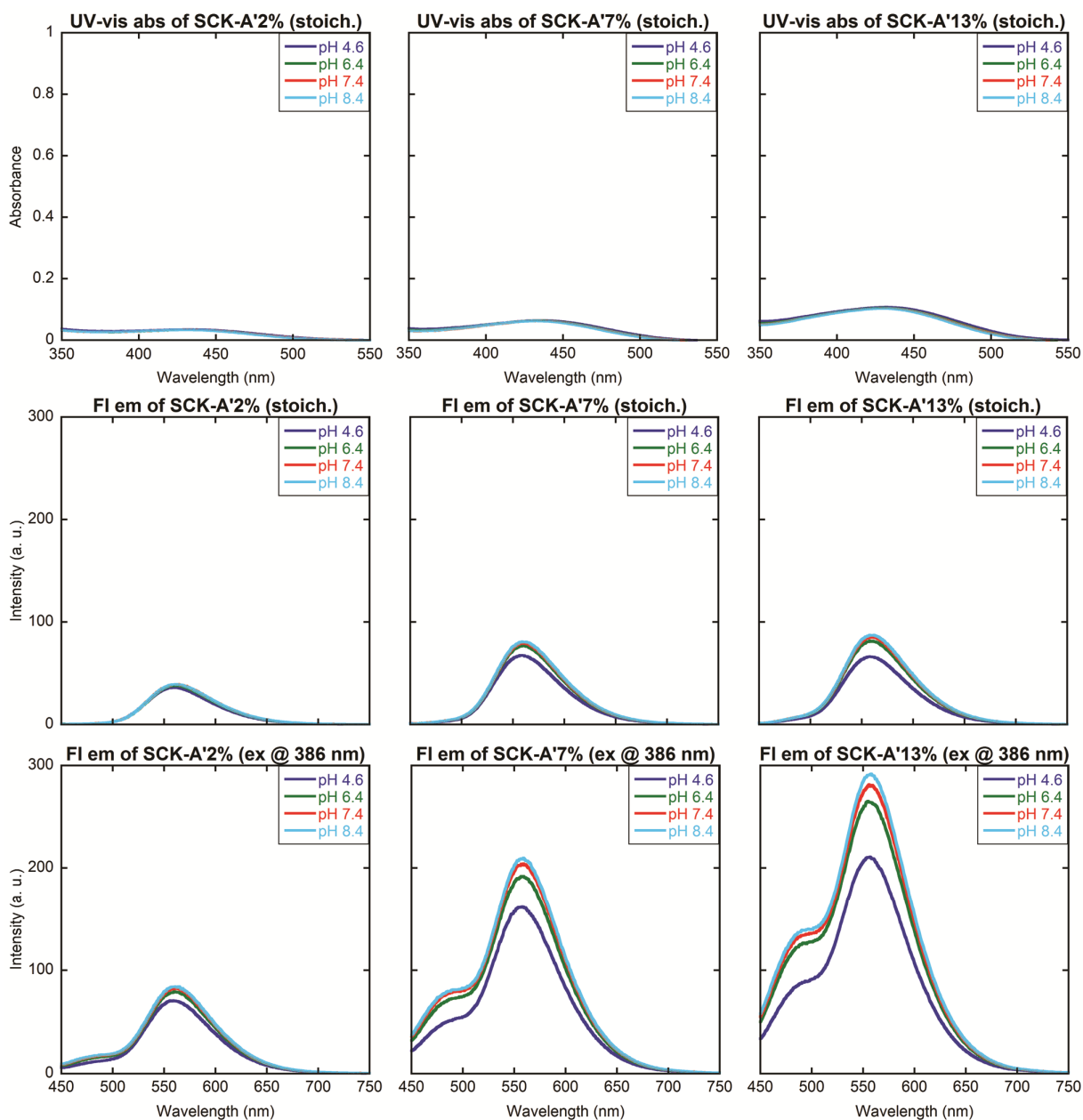


Figure S11. UV-vis absorbance and fluorescence emission spectra of SCK-A at 2%, 7% or 13% crosslinking density with two cycles of shell crosslinking reactions by addition of a stoichiometric amount of EDCI, relative to the aliphatic amines of the crosslinker.

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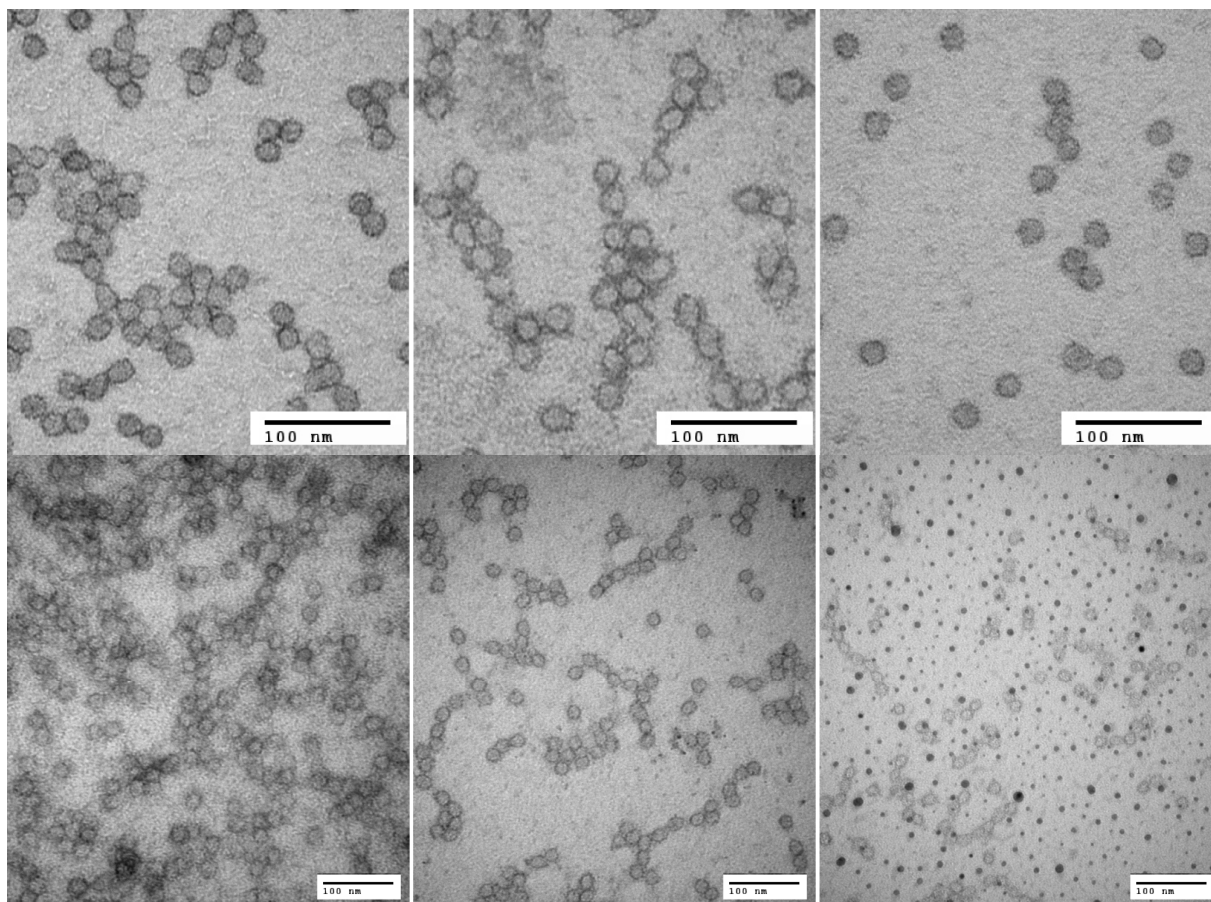


Figure S12. TEM images of sc-SCKs (top row, sc-SCK-As; bottom row, sc-SCK-Bs) in 5 mM of buffer with 5 mM of NaCl at pH 5.8 (left), pH 7.2 (middle), and 8.6 (right), respectively. The images were obtained after negatively stained with PTA.

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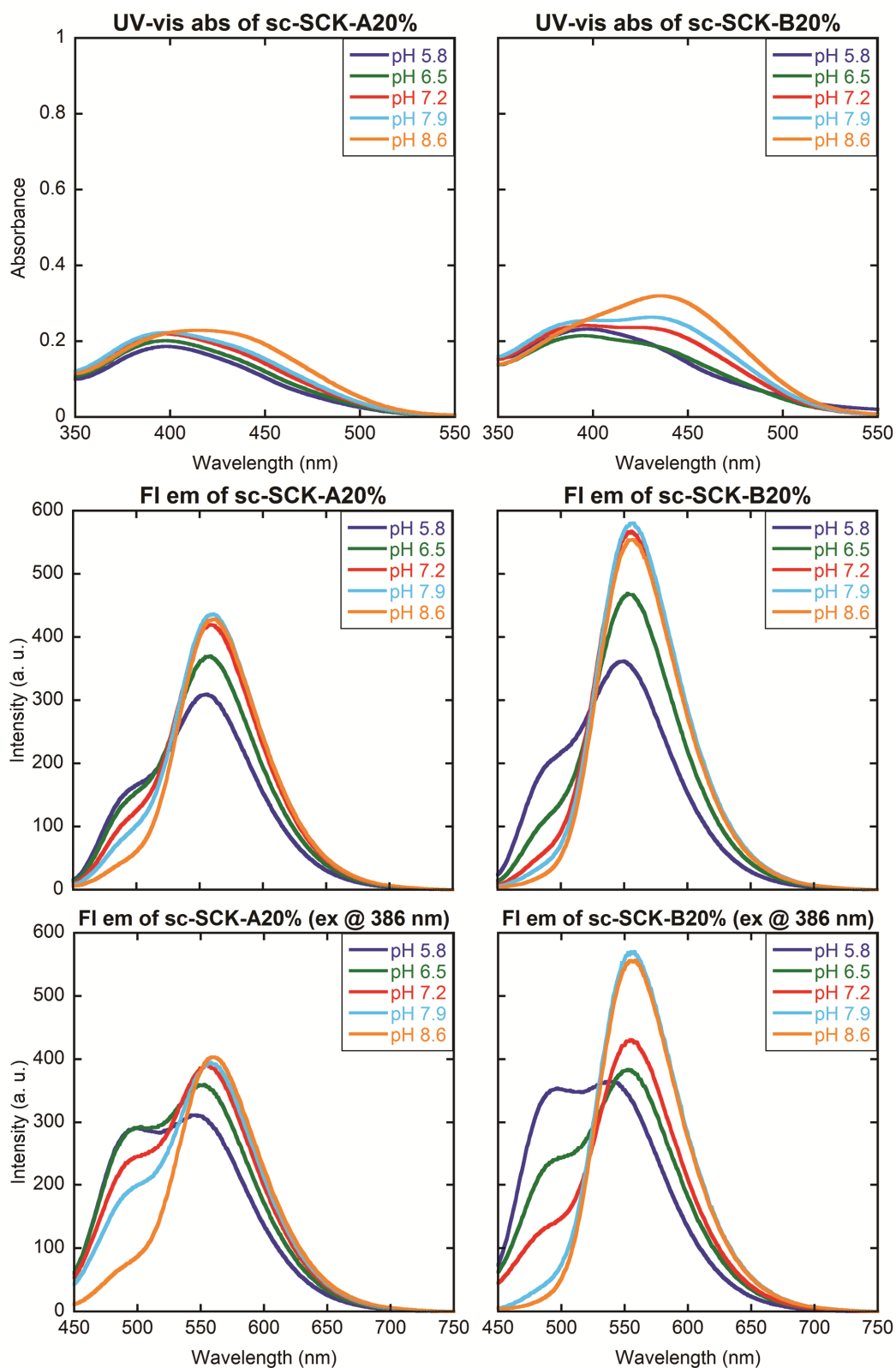


Figure S13. UV-vis absorbance and fluorescence emission spectra of sc-SCK-A and sc-SCK-B at 20% crosslinking density.

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Table S1 Properties of sc-SCKs and lc-SCKs

Samples ^a	Media pH value ^b	$D_{h, \text{intensity}}$ (nm) ^c	D_{av} (nm) ^d	$\lambda_{\text{abs, max}}$ (nm)
sc-SCK-A/lc-SCK-A	5.8	48 ± 4/54 ± 2	18 ± 1/23 ± 2	398/404
sc-SCK-A/lc-SCK-A	6.5	48 ± 4/49 ± 1	ND ^e	398/408
sc-SCK-A/lc-SCK-A	7.2	49 ± 2/49 ± 1	19 ± 2/23 ± 2	398/413
sc-SCK-A/lc-SCK-A	7.9	48 ± 3/52 ± 4	ND ^e	399/418
sc-SCK-A/lc-SCK-A	8.6	49 ± 5/65 ± 3	18 ± 1/23 ± 1	416/421
sc-SCK-B/lc-SCK-B	5.8	48 ± 3/43 ± 3	22 ± 1/26 ± 1	398/413
sc-SCK-B/lc-SCK-B	6.5	49 ± 4/49 ± 2	ND ^e	398/413
sc-SCK-B/lc-SCK-B	7.2	49 ± 2/50 ± 2	21 ± 2/27 ± 2	397/421
sc-SCK-B/lc-SCK-B	7.9	50 ± 2/48 ± 2	ND ^e	431/427
sc-SCK-B/lc-SCK-B	8.6	40 ± 1/52 ± 2	21 ± 2/26 ± 1	436/428

^a Sample concentrations were 0.20-0.30 mg/mL, **SCK2a-2e** and **SCK3a-3e** were prepared by using A and B as cross-linkers, respectively. ^b 5 mM buffer solutions (with 5 mM of NaCl). ^c Intensity-average hydrodynamic diameters with standard deviations between five runs. ^d D_{av} values were measured for the SCK core domains, for at least 100 particles. ^e Not determined.