Supporting Information

Ensemble and Single Particle Fluorescence Characterization of Dye-labeled Cellulose Nanocrystals

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Synthesis of Dye-Labeled CNCs

1 5-(4, 6-dichlorotriazinyl) amino fluorescein (DTAF) labeling of sulfated CNCs. CNC (sulfated) suspension was diluted to 1 wt% and 4 wt% with water, as recommended by Abitbol.[1-3] Solid NaOH and DTAF were added directly to the appropriate volume of CNC suspension (50 mL of 1 wt% and 50 mL of 4 wt%) such that the mass ratio of CNCs to DTAF was constant (1g CNC: 14 mg DTAF). The CNCs were reacted with DTAF under alkaline conditions (0.2 M NaOH) for 24 h in the dark at room temperature temperature (20–22 °C) with stirring. The products of the two reactions were isolated by centrifugation with Millipore 30K MW centrifuge filters at 3175g (4500 RPM) using a 90 minute cycle on a Hettich Universal 320R centrifuge. After centrifugation, the filters were washed once with 0.1 M NaOH solution and the product was resuspended in water. Dialysis was performed against deionized water using a Spectra/Por 4 12–14 kD dialysis membrane. The water was exchanged every 12 hours and the dialysis process was monitored by measuring the absorption of the dialysis water using a Cary 5000 UV-Vis-NIR spectrometer. The dialysis was stopped when the absorbance of the water wash was less than 0.0005 at 490 nm, which required approximately 5 water exchange cycles. The two suspensions were adjusted to pH 6.5 using sulfuric acid and stored in the refrigerator at 4 °C. The photophysical results indicate that the 4 wt% and 1 wt% dye reactions gave labeled CNCs with similar performance and data for only the reaction using 4 wt% is reported (Table 1).

Rhodamine B isothiocyanate (RBITC) labeling of sulfated CNCs. A simple one-pot reaction for labeling microcrystalline cellulose by reaction of free hydroxyl groups with isothiocyanates to form the thiocarbamate bond has been reported[4] and was adapted for RBITC labeling of CNC suspensions as described by Nielsen et al.[5] The never-dried CNC suspension (sulfated) was diluted to 1 wt% with water and reacted with 0.1 M NaOH and a specific amount of RBITC at room temperature in the dark with stirring. Three different reaction times and RBITC concentrations were used to obtain CNCs with different dye loadings (Table 1).

Table S1: RBITC concentration and reaction time for dye labeling reaction

Reaction conditions	CNC-RB-1	CNC-RB-2	CNC-RB-3
[RBITC] (mg/L)	420	45	200
Reaction time (hr)	96	48	72
Sample volume (mL)	100	100	200

The CNCs were isolated by centrifugation with a Millipore 30K MW centrifuge filter and then washed once with 0.1 M NaOH solution using the same filter. The CNC was resuspended and dialysed against Milli-Q water using a Spectra/Por 4 12–14 kD dialysis membrane. The water was changed every 12 hours and the absorbance of the washed water was measured using a Cary 5000 UV-Vis-NIR spectrometer. The dialysis required 3–5 days to obtain an absorbance of the wash water of less than 0.0005 at 555 nm (10 mm path length quartz cell). Finally, the dye-labeled CNC suspension was stored in the refrigerator at 4 °C. The suspension concentrations were estimated gravimetrically after freeze drying.

Lissamine rhodamine B ethylenediamine (LRBED) labeling of carboxylated CNCs. Carboxylated CNCs (100 mg) were dispersed in 5.76 mL MES buffer (0.01M, pH = 4.75) by shaking vigorously for 5 s and then sonicated (probe sonicator with 50% amplitude, total energy of 500 J).[6,7] N-hydroxysuccinimide (6.76 mg) was added to the stirred suspension followed by addition of 3.75 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The pH was maintained at 4.7 and the suspension was mixed for 15 minutes. Then 11.775 mg of LRBED was added to the suspension while stirring. The pH was increased to 7 by adding 1M NaOH and the reaction mixture was stirred for 24 hr. The reaction mixture was dialyzed for 10 days against deionized water (~3.5 L) to remove unreacted reagents. The dialysis medium was changed 3 times per day and the conductivity was monitored to ensure adequate dialysis time. Additional washes were done by centrifugation to completely remove the unattached dyes until no change was observed in the absorbance of the labeled CNC (CNC-RB-4) with further washing. The sample was freeze-dried. Both the never-dried suspension (0.02 wt%) and samples redispersed at 0.14 wt% with 5000 J/g sonication[8] were studied by absorption and fluorescence spectroscopy.

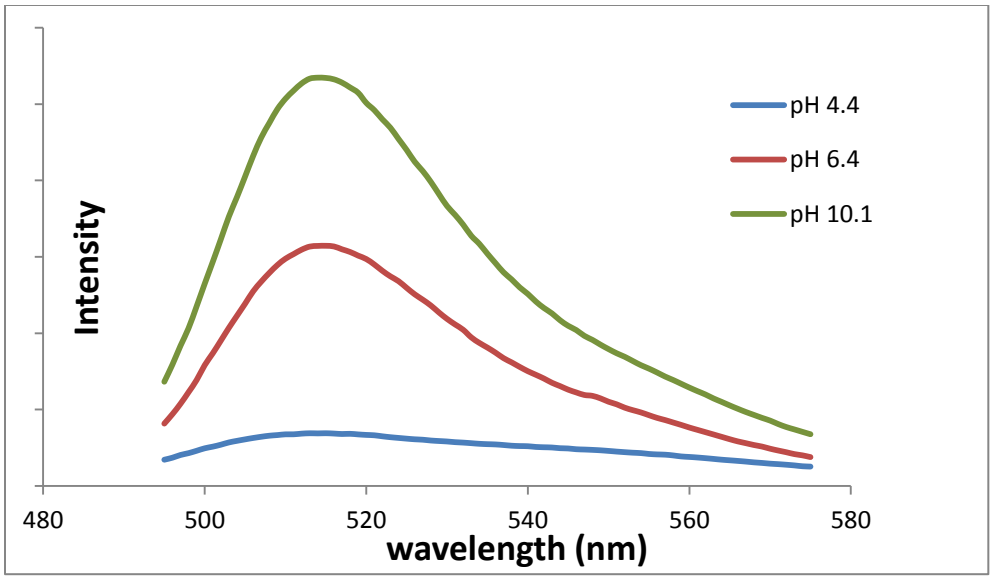

Figure S1. CNC-Fl fluorescence as a function of pH.

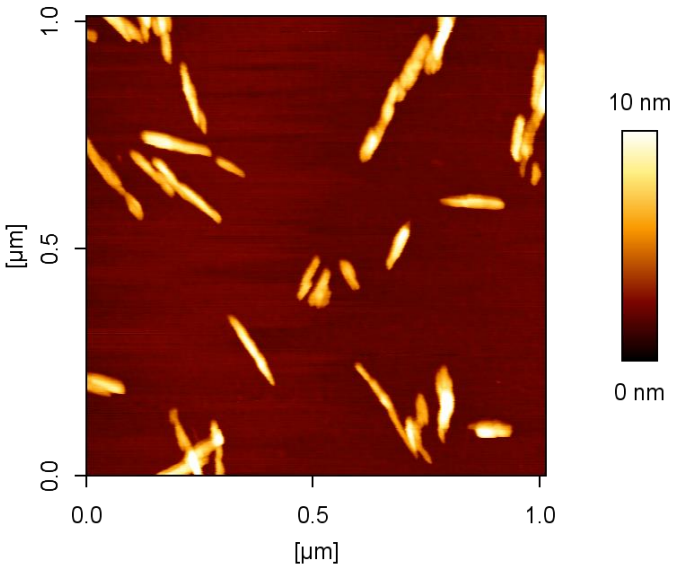

Figure S2. AFM image for CNC-RB-1 imaged dry on poly-L-lysine-coated mica.

Table S2. Dynamic light scattering results for CNC samples

Sample	**Z-Average (nm)**	**PDI**
CNC, unsonicated	140	
CNC, sonicated	66	0.16
RBITC-CNC-1	105	0.203
RBITC-CNC-2	101	0.173

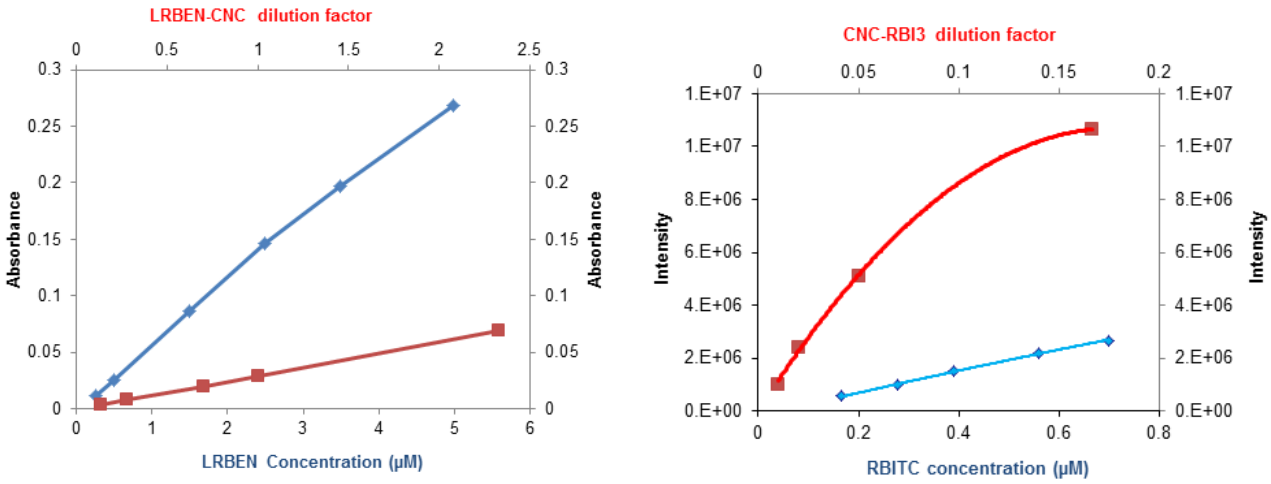

Figure S3. (Left) Plots of absorbance vs concentration and dilution for LRBENand CNC-RB-4, respectively. (Right) Plots of fluorescence intensity vs concentration and dilution for RBITC and CNC-RB-3, respectively. Calculations of fluorescence efficiency were based on the linear portion of the curve.

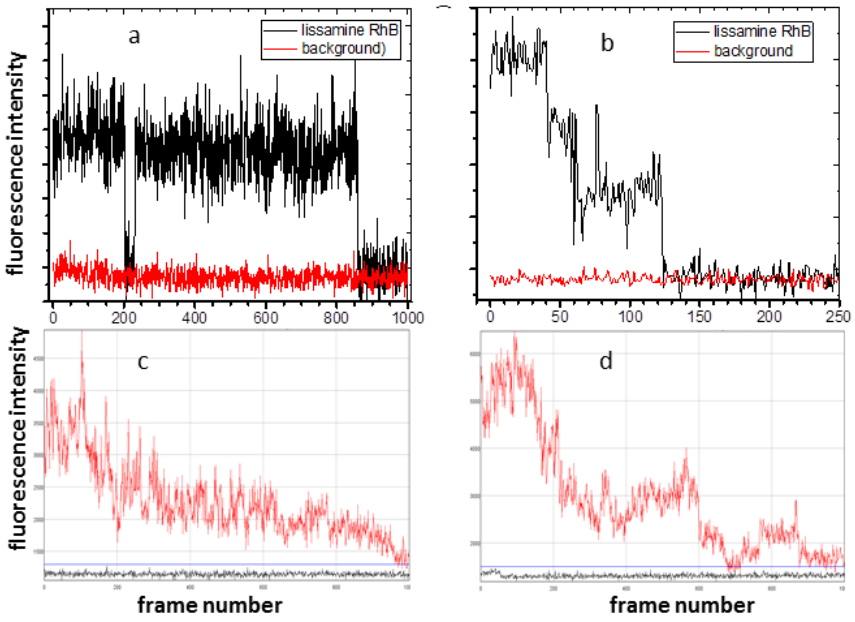

Figure S4. Representative intensity vs time traces for single particle fluorescence for CNC-RB-4.

References

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