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Visualization of nanostructural dislocations in microcrystalline cellulose fibrils through super-resolution fluorescence microscopy

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Cellulose, the primary component of the plant cell wall, is ubiquitous in nature, has fueled the wood, pulp and paper industries for centuries, and has recently been exploited for the production of biofuels and renewable nanomaterials. The tight crystalline packing of glucan chains within cellulose microfibrils is responsible for the superior mechanical properties of this material, but it renders them recalcitrant to biochemical and chemical depolymerization. The presence of dislocations within cellulose microfibrils has been postulated for decades and is thought to be responsible for the production and size of cellulose nanocrystals (CNCs) following the acid hydrolysis or oxidation of cellulose fibers.[1] However, these dislocations have never been directly visualized and their prevalence and size have remained elusive. In this study, we have used super-resolution fluorescence microscopy to visualize, for the first time, crystalline and disordered regions within individual fluorescently labelled bacterial cellulose microfibrils (Figure 1). The distribution of the measured crystalline regions shows striking overlap with the length distribution of bacterial CNCs produced through sulfuric acid hydrolysis. We use the degree of crystallinity, length and width of bacterial CNCs in combination with super-resolution results to elucidate the role of dislocations in the supramolecular structure of bacterial cellulose microfibrils and in the production of CNCs (Figure 2). Results support the model of alternating crystalline and disordered regions where the disordered regions are 50 - 200 nm in size with an average separation of 300 nm. In addition, through two-colour STORM, we simultaneously visualize the distribution of adsorbed cellobiohydrolases (Cel7A) and disordered regions on individual cellulose microfibrils to investigate the role of cellulose dislocations on enzyme binding. Through characterization of disordered regions in cellulose microfibrils, we have gained insight into the role of cellulose nanostructure in its breakdown by chemical and enzymatic means.

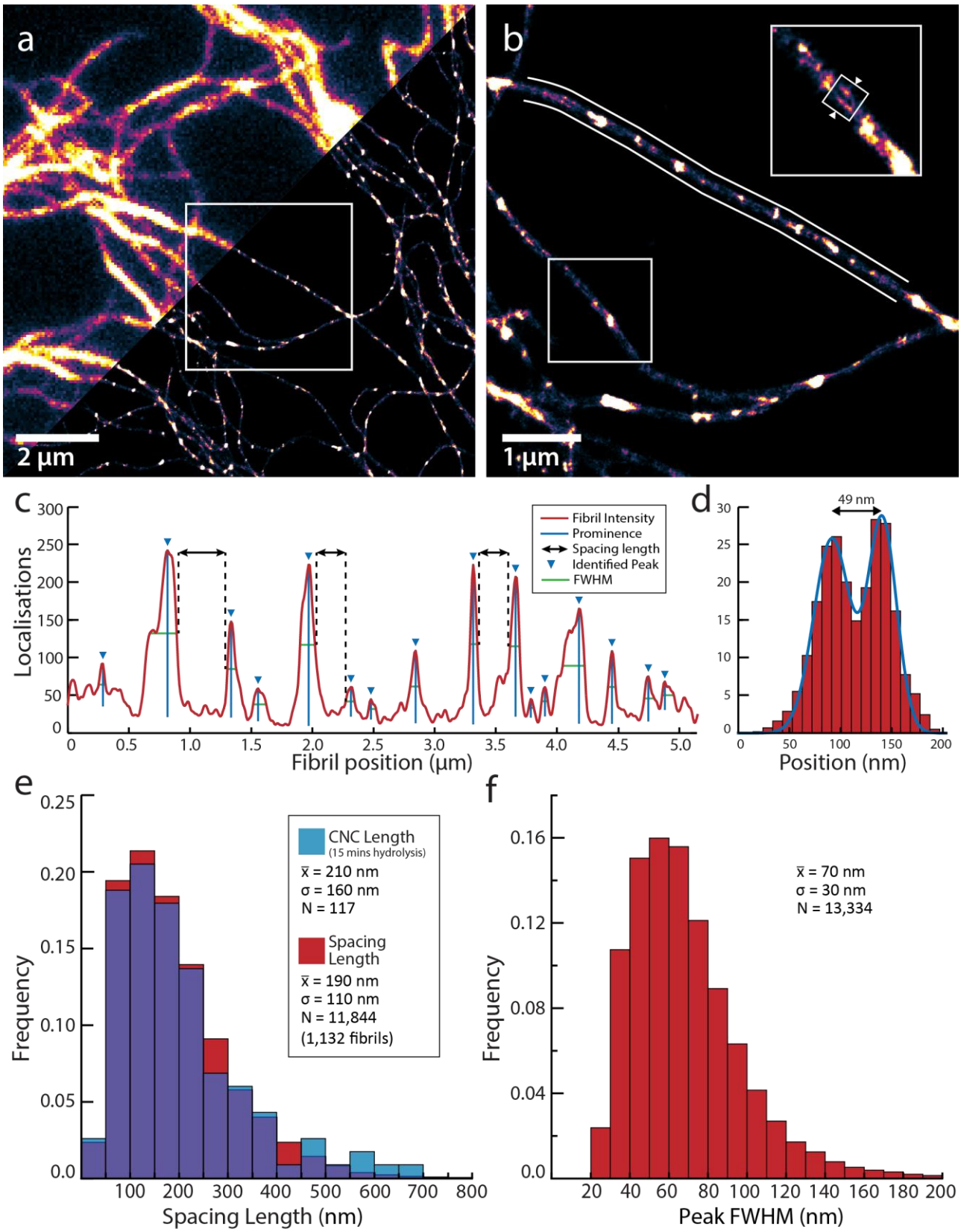

Figure 1. Figure 1. Super-resolution images of ACy5-BC microfibrils and analysis of the labelling pattern. (a) Super-resolution images of ACy5-BC microfibrils unveiled a one-dimensional labeling pattern of alternating dark

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and bright regions that persists throughout the length of the microfibril. STORM imaging yielded average localization uncertainties of ~15 nm. (b) The pattern of repeating dark and bright regions was were analyzed by manually tracing the thinnest cellulose fibrils and acquiring intensity profiles along their longitudinal axis. (c) Intensity profiles evidenced the contrast in labelling densities of some regions on the fiber. The bright peaks were selected and characterized for their full width at half maximum (FWHM) and intensity. (d) Cross-sectional detail of BC microfibrils with sub-50 nm features such as the fibril twisting in the inset of panel b, were occasionally seen in SR images (e) The length of the dark regions was calculated as the distance between adjacent peaks at half their intensity. Their distribution (red,), closely mirrors the length measurements (through TEM) for B-CNCs produced by 15 minutes of acid hydrolysis (teal) with the overlap shown in purple. (f) The distribution of the FWHM of the peaks in comparison was much narrower and at significantly smaller length scales (inset).

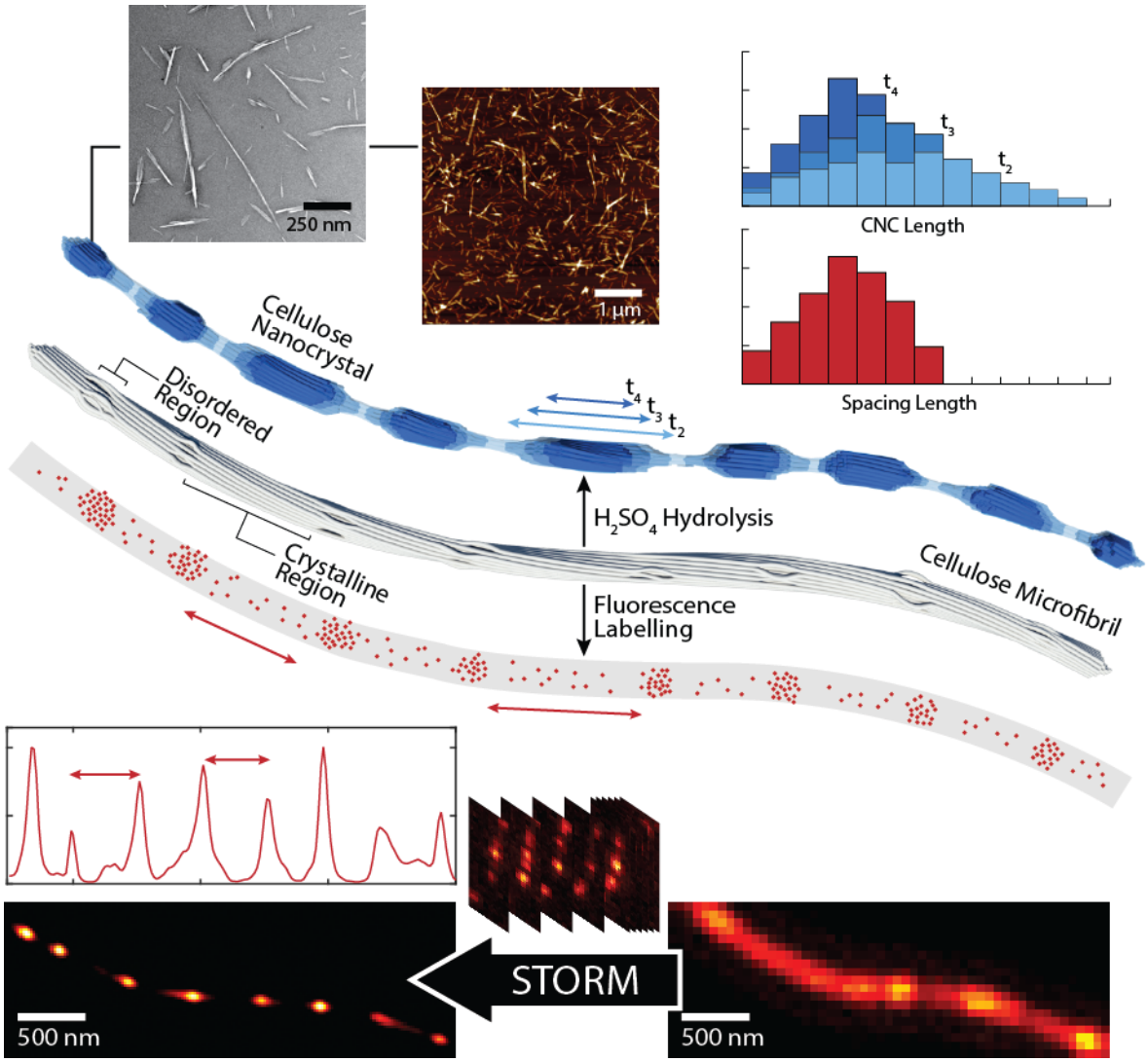

Figure 2. Figure 2. Visualizing dislocations in bacterial cellulose microfibrils using super-resolution fluorescence microscopy. The fringed-micellar structural model of the cellulose microfibril (grey) postulates the presence of alternating regions of disordered and ordered glucan chains along the longitudinal axis. Areas of disorder are suspected to be more accessible to small molecules than those of high crystallinity, thus making them more susceptible to acid hydrolysis (blue) or labelling with fluorescent organic dyes (red). In this model, during acid hydrolysis, the disordered regions are preferentially degraded and eventually the cellulose fibril is cleaved at these regions, producing highly crystalline cellulose nanocrystals (B-CNCs). The length and height of these particles can be measured after different durations of acid hydrolysis using TEM and AFM, respectively. Fluorescent labelling

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of cellulose is expected to create clusters of fluorophores centered around the more accessible (i.e., disordered regions). The enhanced resolution offered by the STORM super-resolution technique yields a repeating pattern of alternating bright and dark segments that correspond to disordered and crystalline regions, respectively. The size of the latter can be determined by measuring the distance between the peaks, and their distribution (red histogram) should correspond to that of the length of B-CNCs produced following acid hydrolysis (blue histogram). For the purpose of clearly showing the glucan dislocations, the microfibril is represented with a significantly lower length to width aspect ratio than in the real system.

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