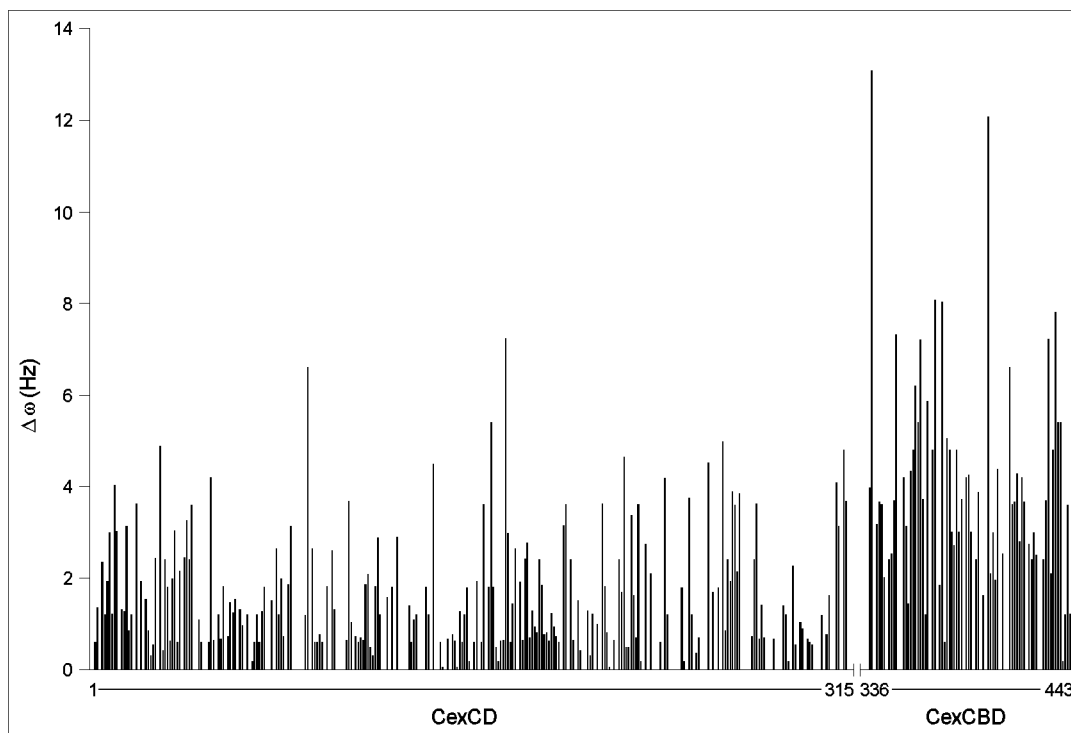
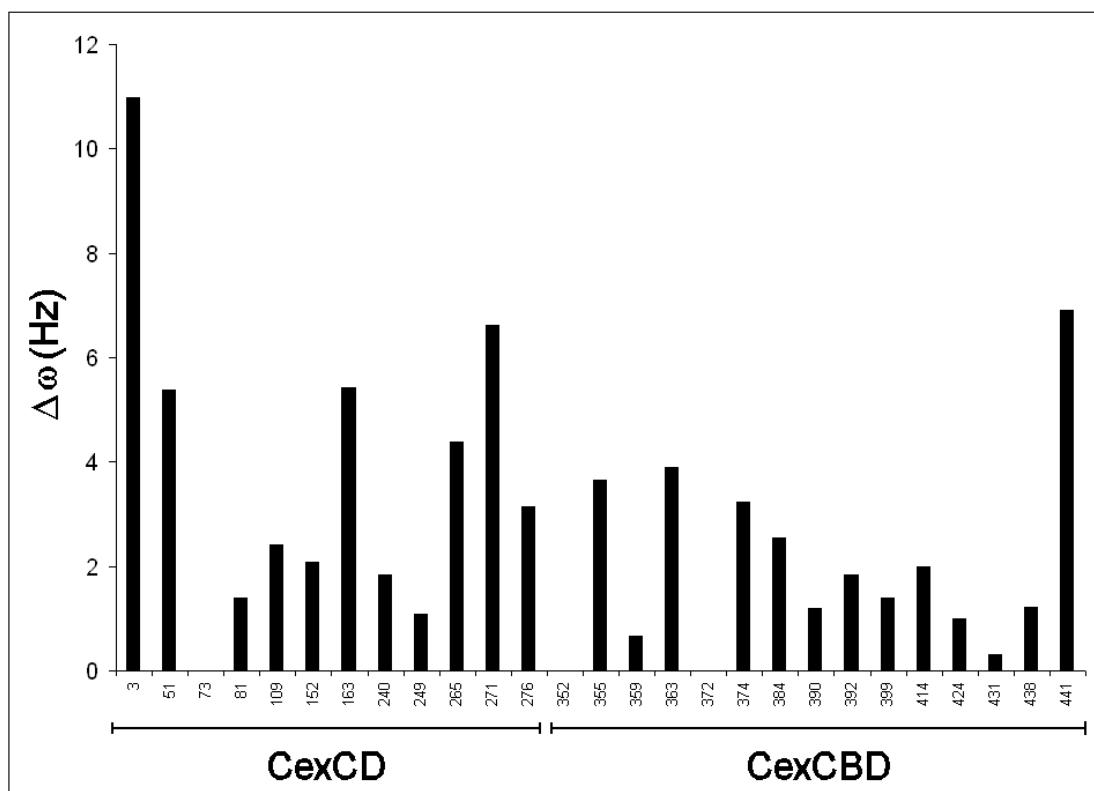


“Direct demonstration of the flexibility of the glycosylated proline-threonine linker in the *Cellulomonas fimi* xylanase Cex through NMR spectroscopic analysis”



**Supplemental Fig. S1.** The modular CexCD and CexCBD domains are structurally independently of each other when tethered by the PT-linker in the native enzyme. This conclusion is supported by the insignificant amide  $^1\text{H}^{\text{N}}$  and  $^{15}\text{N}$  chemical shift differences for corresponding amides in full-length Cex versus the isolated CexCD and CexCBD domains. The shift differences (Hz) are calculated as  $\Delta\omega = [(\Delta\omega_{\text{H}})^2 + (\Delta\omega_{\text{N}})^2]^{1/2}$  from data recorded with a 600 MHz NMR spectrometer. Data corresponding to prolines and overlapped and/or unassigned peaks are not plotted. Note that the typical  $^1\text{H}$  line width of an amide signal in these spectra is  $\sim 35$  Hz with data processed to 5 Hz/point. For comparison, formation of a trapped glycosyl-enzyme intermediate leads to amide chemical shift perturbations in excess of 100 Hz (35).

“Direct demonstration of the flexibility of the glycosylated proline-threonine linker in the *Cellulomonas fimi* xylanase Cex through NMR spectroscopic analysis”



**Supplemental Fig. S2.** Glycosylation does not induce any detectable structural changes in the catalytic and cellulose-binding domains of Cex. This conclusion is supported by the insignificant chemical shift perturbations of the threonine amides in the full length protein upon its glycosylation. The shift differences (Hz) are calculated as  $\Delta\omega = [(\Delta\omega_H)^2 + (\Delta\omega_N)^2]^{1/2}$  from data recorded with a 600 MHz NMR spectrometer. Data corresponding to overlapped and/or unassigned peaks, including those of the PT-linker threonines, are not plotted. Note that the typical  $^1\text{H}$  line width of an amide signal in these spectra is  $\sim 35$  Hz with data processed to 5 Hz/point. For comparison, formation of a trapped glycosyl-enzyme intermediate leads to amide chemical shift perturbations in excess of 100 Hz (35).