

Supplemental material to

Multi-mode binding of cellobiohydrolase Cel7A from *Trichoderma reesei* to cellulose

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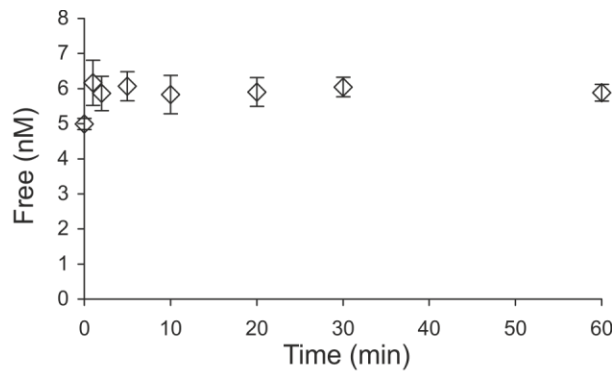


Figure S1. Release of *TrCel7A* from cellulose pellet after centrifugation. BC (0.1 g/L) was incubated with 30 nM *TrCel7A* for 10 min. BC with bound *TrCel7A* was pelleted by centrifugation (2 min at 10,000 g) and the concentration of free *TrCel7A* in supernatant was measured at different times after centrifugation. For zero time point the cellulose bound and free *TrCel7A* was separated by filtration.

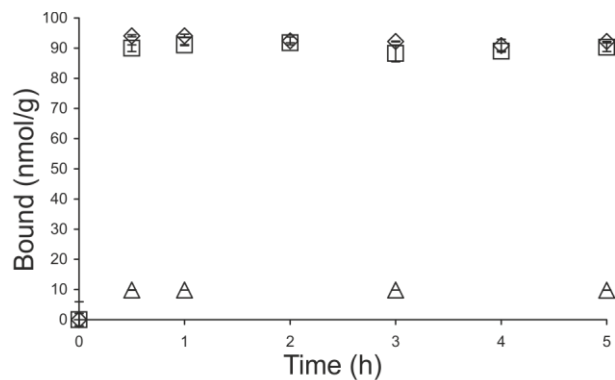


Figure S2. Binding kinetics at different concentrations of *TrCel7A* and BC. 0.1 μM *TrCel7A* and 1.0 g/L BC (◇), 0.01 μM *TrCel7A* and 0.1 g/L BC (□), and 0.01 μM *TrCel7A* and 1.0 g/L BC (△).

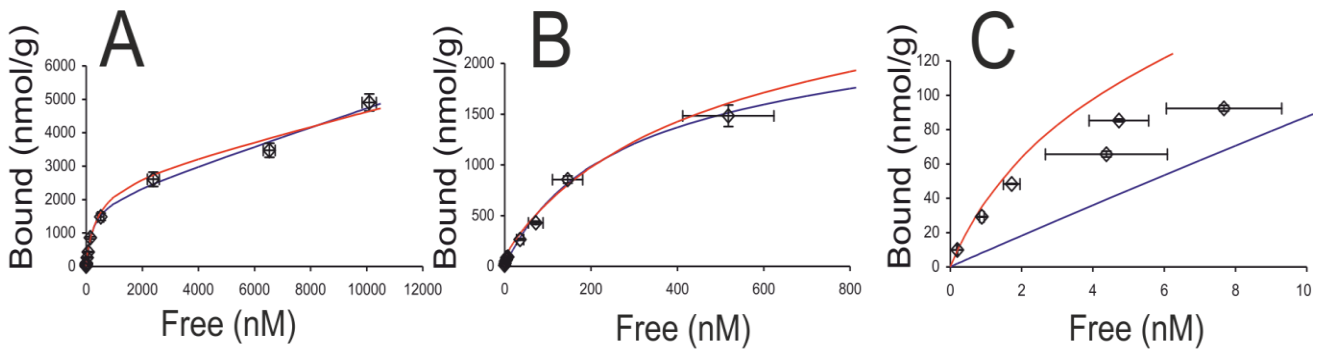


Figure S3. The presence of at least three binding sites must be assumed to describe the binding of *TrCel7A* to BC. Binding isotherm is dissected (as in Figure 1 of the main article) to show the regions with low (A), medium (B), and high (C) affinity dominating binding modes. Solid lines represent the best fit according to the Langmuir's two (blue line) and three (red line) binding sites model. Although the two sites model is sufficient to describe the binding in the low (A) and medium (B) affinity region, it fails to account for the high affinity binding (C). The presence of at least three binding modes was further confirmed by distinguishing between different populations of bound enzyme (Figure 1 of main article).

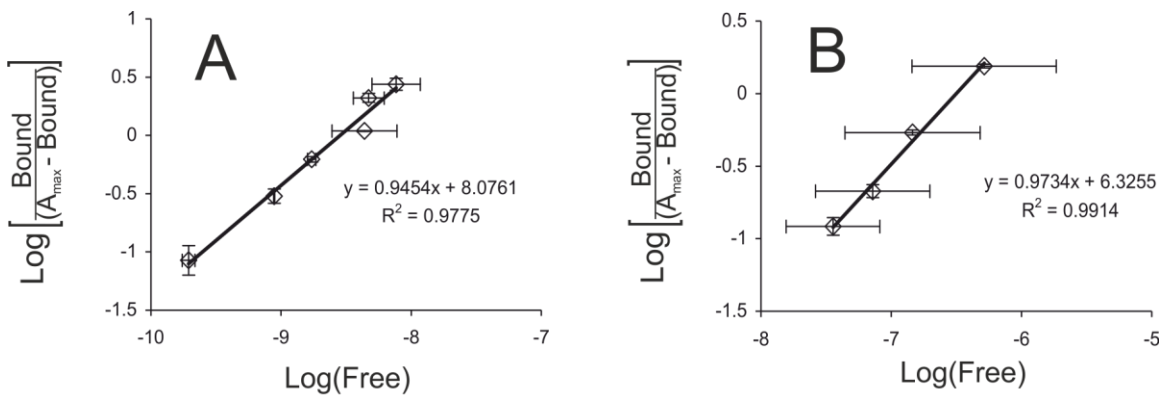


Figure S4. Hill plots for the binding of *TrCel7A* to BC in the high (A) and medium (B) affinity binding mode. The A_{max} values of 126 nmol/g and 2440 nmol/g were used to construct Hill plots for high and medium affinity binding mode, respectively. Solid lines represent the best fit according to the linear function. For both binding mode the slope of the line is close to 1 indicating no cooperativity within these binding modes.

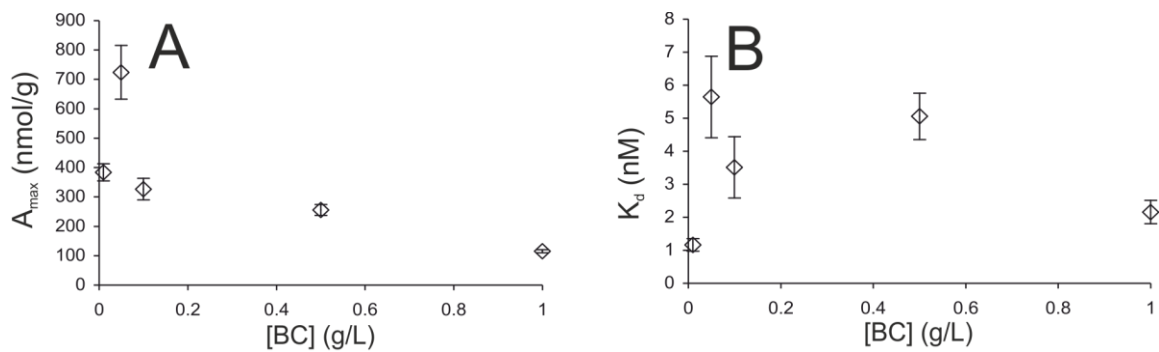


Figure S5. A_{\max} and K_d values for the binding of *TrCel7A* in the high affinity binding mode at different BC concentrations. Binding data (original data are presented in panel A of Figure 4 in the main article) were analyzed according to the Langmuir's one binding site model to get the A_{\max} and K_d values.