Supplementary data

Endo-exo synergism in cellulose hydrolysis revisited*

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*Running title: *Synergism in cellulose hydrolysis*

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Hydrolysis of ^{14}C-BC by *Tr*Cel7A under single-turnover conditions

Table S1 Values of kinetic parameters for *Tr*Cel7A from single-turnover experiments at different *Tr*Cel7A concentrations and at different times of trap addition

[*Tr*Cel7A]*	Trap time†	$[^{14}CB]_{max}$‡	$[ES]_{trap}$§	P^{app}¶	k‡	k_{cat}‖
µM	s	µM	µM	CB units	s^{-1}	s^{-1}
0.1	30	4.5 ± 1.3	0.082 ± 0.003	54 ± 15	0.029 ± 0.059	1.5 ± 3.6
0.25	10	7.0 ± 0.4	0.12 ± 0.02	61 ± 11	0.035 ± 0.007	2.1 ± 0.4
0.5	10	11 ± 0.5	0.16 ± 0.02	69 ± 8	0.044 ± 0.005	3.1 ± 0.4
1.0	10	15 ± 2	0.22 ± 0.10	70 ± 33	0.031 ± 0.006	2.2 ± 0.7
1.0	30	20 ± 1	0.28 ± 0.11	70 ± 28	0.026 ± 0.014	1.8 ± 1.0
1.0	60	23 ± 3	0.34 ± 0.12	69 ± 25	0.032 ± 0.023	2.2 ± 1.6
Average				66 ± 7	0.033 ± 0.006	2.2 ± 0.5

* Experiment conditions were: [^{14}C-BC] = 0.5 mg ml^{-1}, [BG] = 0.125 µM, 25ºC, pH 5.0.
†Time of the addition of AC trap after initiation of the hydrolysis of ^{14}C-BC.
‡Values of $[^{14}CB]_{max}$ and k were found by non-linear regression of [^{14}CB] released under single-turnover conditions according to Equation 1.
§Concentration of *Tr*Cel7A at the moment of trap addition ($[ES]_{trap}$) represents the population of *Tr*Cel7A with the active site occupied by the cellulose chain ($[TrCel7A]_{OA}$) at the moment of trap addition (trap time).
¶Apparent processivity of *Tr*Cel7A (P^{app}, in cellobiose units) was found from the values of $[^{14}CB]_{max}$ and $[ES]_{trap}$ according to Equation 3.
‖The value of k_{cat} was found from the values of k and P^{app} according to Equation 5.

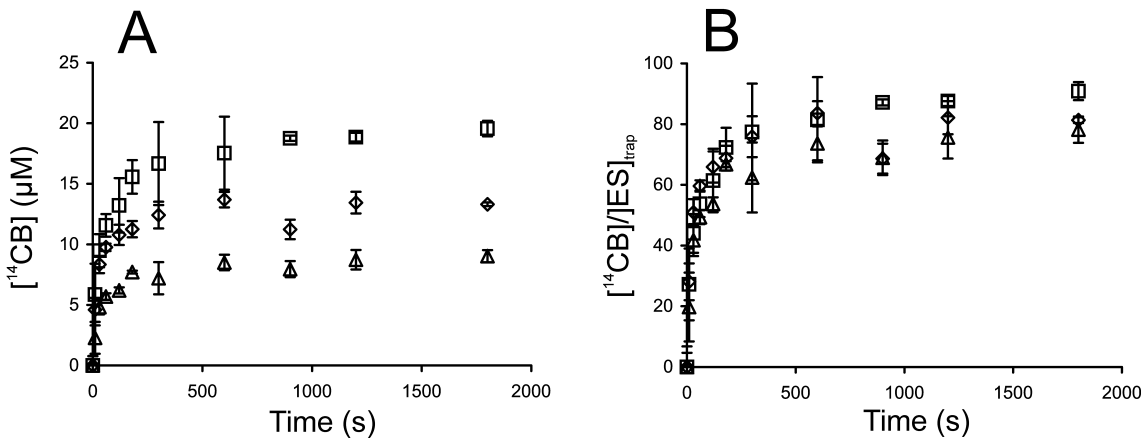

Figure S1. Hydrolysis of ^{14}C-BC under single-turnover conditions at different *Tr*Cel7A concentrations. [^{14}C-BC] = 0.5 mg ml^{-1}, [β-glucosidase] = 0.125 μM, 25ºC, pH 5.0. [*Tr*Cel7A] was 0.25 μM (△), 0.5 μM (◇), or 1.0 μM (□). AC trap was added after 10 s of hydrolysis. *A.* Formation of [^{14}CB] upon hydrolysis of ^{14}C-BC with *Tr*Cel7A at different concentrations. *B.* The values of [^{14}CB] from panel A were divided with corresponding $[ES]_{trap}$. Concentration of *Tr*Cel7A with the active site occupied by the cellulose chain ($[TrCel7A]_{OA}$) after 10 s of hydrolysis (Fig. 1*B* in main article) was used for $[ES]_{trap}$. For numerical values of $[ES]_{trap}$ refer to Table S1.

Binding kinetics of *Tr*Cel7A to BC

Table S2 Parameters of binding kinetics of *Tr*Cel7A to BC (0.5 mg ml^{-1})

Enzyme(s)	[*Tr*Cel7A] [a]	$[TrCel7A]_{OA\text{-}max}$ [b]	k_{on}^{obs} [b]
	μM	μM	s^{-1}
*Tr*Cel7A [c]	0.1	0.086 ± 0.004	0.155 ± 0.058
	0.25	0.155 ± 0.015	0.125 ± 0.032
	0.5	0.250 ± 0.031	0.095 ± 0.039
	1.0	0.319 ± 0.118	0.096 ± 0.056
*Tr*Cel7A+ EG [d]	1.0	0.808 ± 0.013	0.165 ± 0.005
$CD_{TrCel7A}$ + EG [d]	1.0	0.427 ± 0.061	0.091 ± 0.019

[a] Total concentration of *Tr*Cel7A. If present the concentration of EG (*Tr*Cel5A) was 0.1 μM.
[b] The values of parameters, $[TrCel7A]_{OA\text{-}max}$ and k_{on}^{obs} were found by non-linear regression according to Equation 2 (main article).
[c] Original data used in non-linear regression analysis are given in main article (Fig. 1*B*).
[d] Original data used in non-linear regression analysis are given in main article (Fig. 2*B*).

Synergism between endoglucanase (EG) and cellobiohydrolase (CBH)

Table S3 Degree of synergistic effect (DSE) between CBH *Tr*Cel7A and EG *Tr*Cel5A in hydrolysis of lignocellulose (hydrothermally pre-treated wheat straw)

	*Tr*Cel7A and *Tr*Cel5A [a]			$CD_{TrCel7A}$ and *Tr*Cel5A [a]		
PWS	DSE_{Glc} [b]	DSE_{OA} [c]	DSE_k [d]	DSE_{Glc} [b]	DSE_{OA} [c]	DSE_k [d]
mg ml^{-1}						
1.0	3.9 ± 0.6	1.3 ± 0.24	3.0 ± 0.6	2.2 ± 0.5	0.9 ± 0.14	2.4 ± 0.6
3.0	3.0 ± 0.7	1.1 ± 0.08	2.7 ± 0.6	2.8 ± 0.3	1.1 ± 0.08	2.6 ± 0.3
5.0	3.3 ± 0.1	1.0 ± 0.03	3.4 ± 0.1	2.4 ± 0.2	1.0 ± 0.05	2.4 ± 0.2
7.5	3.2 ± 0.1	1.0 ± 0.05	3.3 ± 0.2	2.6 ± 0.1	1.1 ± 0.10	2.3 ± 0.2
10	2.9 ± 0.2	1.0 ± 0.03	2.9 ± 0.2	2.4 ± 0.2	1.1 ± 0.03	2.3 ± 0.2

[a] Experiment conditions were: [*Tr*Cel7A] or [$CD_{TrCel7A}$] = 2.5 µM, [β-glucosidase] = 0.85 µM, [pNPL] = 0.5 mM, 25ºC, pH 5.0. If present the concentration of EG (*Tr*Cel5A) was 0.25 µM. Samples were withdrawn after 30 min of hydrolysis and analyzed for glucose formation and $[TrCel7A]_{OA}$.
[b] DSE on the level of glucose formation (DSE_{Glc}) represents the ratio of glucose produced by the mixture of *Tr*Cel7A and *Tr*Cel5A to glucose produced by individual *Tr*Cel7A. Low levels of glucose produced by individual 0.25 µM *Tr*Cel5A were not accounted for in calculating DSE_{Glc}.
[c] DSE on the level of $[TrCel7A]_{OA}$ (DSE_{OA}) represents the ratio of $[TrCel7A]_{OA}$ measured for the mixture of *Tr*Cel7A and *Tr*Cel5A to $[TrCel7A]_{OA}$ measured for individual *Tr*Cel7A. Note that because of the adsorption of pNP to the lignocellulose these figures must be treated with caution.
[d] DSE on the level of observed rate constant (k^{obs}) (DSE_k) represents the ratio of k^{obs} measured for the mixture of *Tr*Cel7A and *Tr*Cel5A to k^{obs} measured for individual *Tr*Cel7A. Note that because of the adsorption of pNP to the lignocellulose these figures must be treated with caution.

Cellobiose inhibition of *Tr*Cel7A under single-turnover conditions

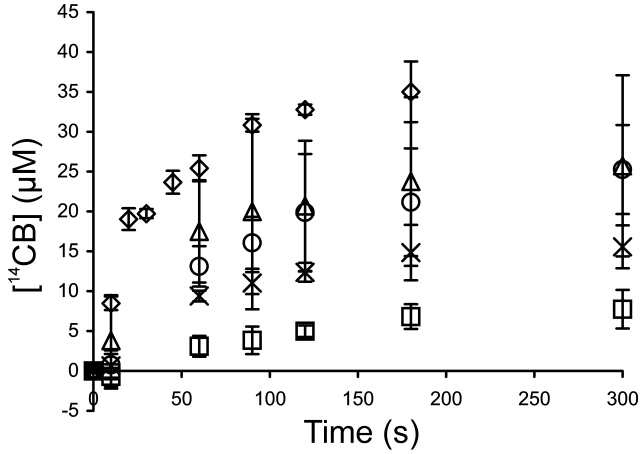

Figure S2 Cellobiose (CB) inhibition of the hydrolysis of ^{14}C-bacterial cellulose (^{14}C-BC) by the mixture of *Tr*Cel7A and EG, *Tr*Cel5A under single-turnover conditions. Concentrations of ^{14}C-BC, *Tr*Cel7A and *Tr*Cel5A were 0.5 mg ml^{-1}, 1.0 µM and 0.1 µM respectively. Series without added cellobiose were provided with 0.125 µM β-glucosidase. AC trap was added after 10 s of hydrolysis. Concentration of added cellobiose was 0 mM (◇), 0.5 mM (△), 2.0 mM (○), 5.0 mM (×), or 20 mM (□).

Table S4 Cellobiose inhibition of *Tr*Cel7A under single-turnover conditions

	Individual *Tr*Cel7A*		*Tr*Cel7A + EG*	
Cellobiose	$[^{14}CB]_{max}$ †	k †	$[^{14}CB]_{max}$ †	k †
mM	μM	s^{-1}	μM	s^{-1}
0	19.9 ± 0.8	0.034 ± 0.010	33.5 ± 1.8	0.030 ± 0.005
0.5	12.2 ± 0.8	0.019 ± 0.004	25.1 ± 10.9	0.018 ± 0.009
2	8.9 ± 1.9	0.023 ± 0.001	25.9 ± 7.3	0.011 ± 0.005
5	8.0 ± 1.3	0.014 ± 0.004	16.0 ± 3.8	0.013 ± 0.005
20	4.4 ± 0.8	0.011 ± 0.004	9.4 ± 1.2	0.006 ± 0.004

* Concentrations of ^{14}C-BC and *Tr*Cel7A were 0.5 mg ml^{-1} and 1.0 μM respectively. If present the concentration of EG (*Tr*Cel5A) was 0.1 μM. Series without added cellobiose were provided with 0.125 μM β-glucosidase. AC trap was added 10 s (for *Tr*Cel7A +EG) or 30 s (for individual *Tr*Cel7A) after initiation of the hydrolysis of ^{14}C-BC.

† Values of $[^{14}CB]_{max}$ and k were found by non-linear regression of $[^{14}CB]$ released under single-turnover conditions according to Equation 1 (main article).

CB inhibition of *Tr*Cel7A in "steady state"

Table S5 Example of the calculation of average $[^{14}CB]_{CB}/[^{14}CB]_{CB=0}$ values. Data are from the hydrolysis of ^{14}C-BC (0.25 mg ml^{-1}) by the mixture of $CD_{TrCel7A}$ (0.25 μM) and *Tr*Cel5A (0.025 μM) in the presence of added cellobiose (CB) at various concentrations. For the original data see supplemental Fig. S3*B*.

	$[^{14}CB]_{CB}/[^{14}CB]_{CB=0}$ [a]					
Time (min)	CB = 0.2 mM	CB = 0.5 mM	CB = 1.0 mM	CB = 2.0 mM	CB = 5.0 mM	CB = 10 mM
10	0.58	0.42	0.37	0.28	0.14	0.09
30	0.63	0.45	0.42	0.25	0.18	0.10
45	0.62	0.43	0.43	0.24	0.14	0.10
60	0.67	0.48	0.45	0.27	0.17	0.10
90	0.65	0.45	0.45	0.28	0.18	0.10
120	0.69	0.49	0.44	0.28	0.17	0.11
Average	0.64	0.45	0.43	0.26	0.16	0.10
STDEV	0.04	0.03	0.03	0.02	0.02	0.01

[a] Time courses of the hydrolysis of ^{14}C-BC were rearranged to obtain the ratio of $([^{14}CB]_{CB}/[^{14}CB]_{CB=0})$ for each time point. $[^{14}CB]_{CB}$ and $[^{14}CB]_{CB=0}$ are the concentrations of released ^{14}CB in the experiments with and without added cellobiose respectively. The ratio of $[^{14}CB]_{CB}/[^{14}CB]_{CB=0}$ at certain concentration of added CB was first found for each time point and Fig. 6 *C* & *D* (main article) plots average values of $[^{14}CB]_{CB}/[^{14}CB]_{CB=0}$ taken over all time points.

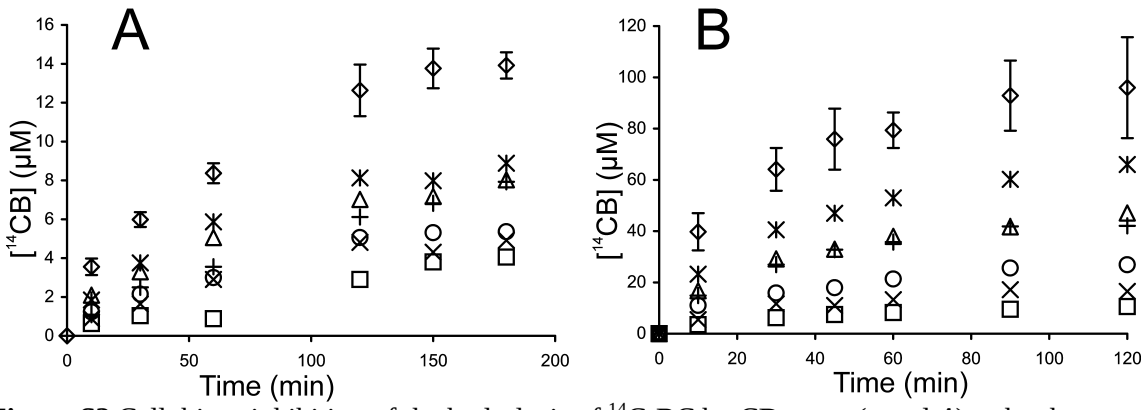

Figure S3 Cellobiose inhibition of the hydrolysis of ^{14}C-BC by $CD_{TrCel7A}$ (panel *A*) or by the mixture of $CD_{TrCel7A}$ and *Tr*Cel5A (panel *B*). Concentrations of ^{14}C-BC and $CD_{TrCel7A}$ were 0.25 mg ml^{-1} and 0.25 μM respectively. If present the concentration of *Tr*Cel5A was 0.025 μM. Series without added cellobiose were provided with 0.125 μM β-glucosidase. Concentration of added cellobiose was 0 mM (◇),0.2 mM (*), 0.5 mM (△), 1.0 mM (+), 2.0 mM (○), 5.0 mM (×), or 10 mM (□).

Calculation of average IC_{50} values for individual CBHs

For the cellobiose inhibition of individual *Tr*Cel7A or its catalytic domain an obvious systematic deviation was observed in fitting the data to Equation 8 (main article). The simplest equation that gave a satisfactory fit without systematic deviation was the one that accounts for the inhibition of the mixture of two enzymes that have the same reaction product and same substrate. Equivalently one can assume one enzyme that uses two alternative modes of action in parallel whereas these modes of action are inhibited with different strengths. In this case the inhibition should be described by the Equation S1:

$$\frac{[^{14}CB]_{CB}}{[^{14}CB]_{CB=0}} = \frac{\dfrac{1}{[S]+K_{M1}\left(1+\dfrac{[CB]}{K_{i1}}\right)}+\dfrac{\dfrac{V_2}{V_1}}{[S]+K_{M2}\left(1+\dfrac{[CB]}{K_{i2}}\right)}}{\dfrac{1}{[S]+K_{M1}}+\dfrac{\dfrac{V_2}{V_1}}{[S]+K_{M2}}} \qquad \text{(Eq. S1)}$$

Where [CB] is cellobiose concentration (mM), [S] is cellulose concentration (mg ml^{-1}), V_1 and V_2, K_{M1} and K_{M2}, and K_{i1} and K_{i2} are limiting rates, Michaelis constants for cellulose (mg ml^{-1}) and inhibition constants for cellobiose (mM) for two modes of action (designated with subscripts 1 and 2) respectively. In non-linear regression analysis the value of [S] was fixed to a value used in the experiments. The values of V_1, V_2, K_{M1}, K_{M2}, K_{i1}, and K_{i2} were obtained from the fitting of the data to Equation S1. These parameter values were further used to calculate the values of IC_{50}. IC_{50} values were found numerically using the definition that if [CB] = IC_{50} then $([^{14}CB]_{CB}/[^{14}CB]_{CB=0}) = 0.5$.

Table S6 IC_{50} values for cellobiose (CB) inhibition of *Tr*Cel7A in "steady state" at different *Tr*Cel7A concentrations

Enzyme(s)*	[*Tr*Cel7A]	IC_{50} for CB†
	μM	mM
*Tr*Cel7A + EG	2.5	0.87 ± 0.18
	0.25	0.38 ± 0.03
	0.025	0.33 ± 0.06
*Tr*Cel7A	0.25	0.65 ± 0.15
	0.020	0.60 ± 0.30

* Concentration of ^{14}C-BC was 0.25 mg ml^{-1}. If present the concentration of EG (*Tr*Cel5A) was 0.025 μM.
† IC_{50} values for cellobiose were found using non-linear regression of the data in coordinates ($[^{14}CB]_{CB}/[^{14}CB]_{CB=0}$) *versus* [CB] according to the supplemental Equation S1 (for individual *Tr*Cel7A) or Equations 8 – 9 in main article (for *Tr*Cel7A +EG).