SUPPORTING INFORMATION FOR

Characterisation of the Effect of the Spatial Organisation of Hemicellulases on the Hydrolysis of Plant Biomass Polymer

Thomas Enjalbert [1], Marion De La Mare [2], Pierre Roblin [3], Louise Badruna [1], Thierry Vernet [4], Claire Dumon [1] and Cédric Y. Montanier [1,*]

[1] Toulouse Biotechnology Institute (TBI), Université de Toulouse, CNRS, INRAE, INSA, 31077 Toulouse, France; enjalber@insa-toulouse.fr (T.E.); louise.badruna@hotmail.fr (L.B.); cdumon@insa-toulouse.fr (C.D.)

[2] Toulouse White Biotechnology, UMS INRA 1337, UMS CNRS 3582, Institut National des Sciences Appliquées de Toulouse, 31077 Toulouse, France; mdelamare@enobraq.com

[3] Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, 31077 Toulouse, France; roblin@chimie.ups-tlse.fr

[4] Institut de Biologie Structurale, Univ., Grenoble Alpes, CEA, CNRS, IBS, F-38000 Grenoble, France; thierry.vernet@ibs.fr

* Correspondence: cedric.montanier@insa-toulouse.fr; Tel.: +33-(0)5-61-55-97-13

This supplementary Information contains 2 sections:

1. Figure S1 to S8
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1. Figure S1 to S8

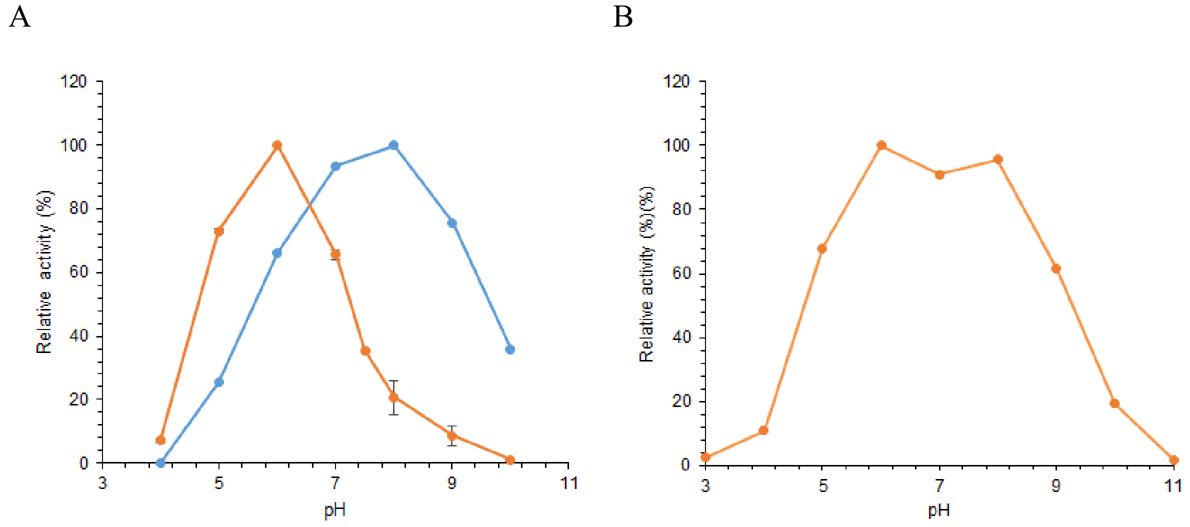

Figure S1. Effect of pH on activity of **A)** His-In-*Np*Xyn11A (orange, from Montanier *et al*.[1]) and His-Jo-*Bh*Xyl43 (blue, this work). Enzyme activity was measured in a pH range between 4 and 10 using 5 mM of 4-nitrophenyl-β-D-xylotrioside at 37 °C and 5 mM of 4-nitrophenyl-β-D-xylopyranoside at 45 °C, respectively. **B)** His-In-*Np*Xyn11A. Enzyme activity was measured in a pH range between 3 and 11 using 2% Beech wood xylan mM at 37 °C.

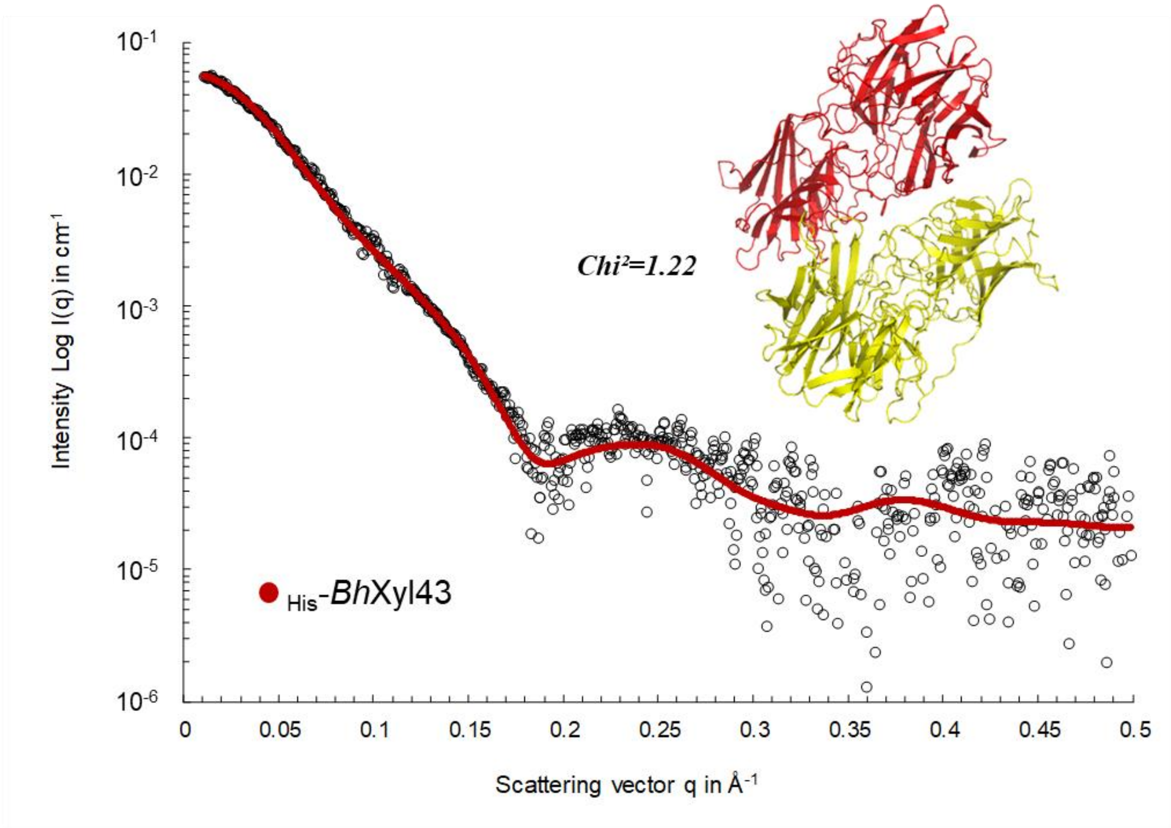

Figure S2. Superimposition of SAXS data from the beta-1,4-xylosidase His-*Bh*Xyl43 plotted with dark dots and the SAXS curve computed from the crystallographic structure of the tetramer (PDB: 1YRZ) plotted in red line. The data are presented as a plot of Log I(q) vs. q with the intensity I(q) in cm-1 and scattering vector q in $Å^{-1}$.

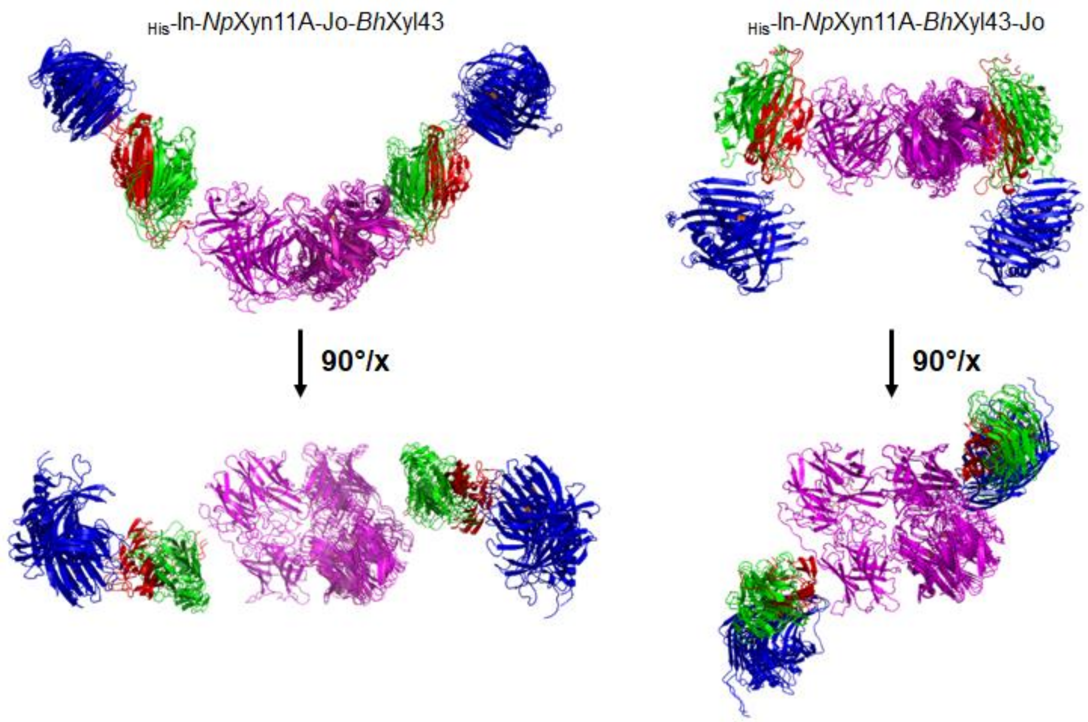

Figure S3. SAXS data compatible models of His-In-*Np*Xyn11A-Jo-*Bh*Xyl43 and His-In-*Np*Xyn11A-*Bh*Xyl43-Jo shown in two orientations. The three extreme structures from the set with a good fit are superimposed for both constructs. Domain *Np*Xyn11A is in blue, domain *Bh*Xyl43 is in magenta, domain In is in green and domain Jo is in red.

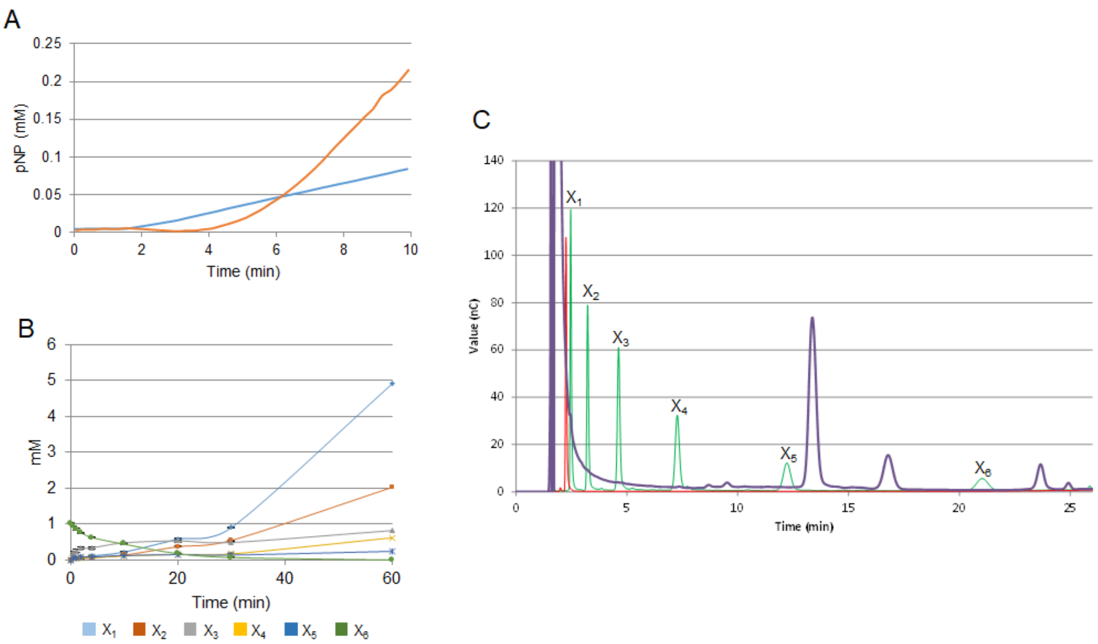

Figure S4. Activity of His-*Bh*Xyl43 on small oligosaccharides. (A) Activity of 8 nM of His-*Np*Xyn11A (blue) and His-*Bh*Xyl43 (red) over 5 mM of *p*NP-X_3 in 50 mM phosphate pH 7 supplemented with 1 mg/ml of BSA, at 37 °C, follow by the release of *p*NP at 401 nm. (B) HPAEC-PAD analysis of the hydrolysis of 1 mM xylohexaose with 60 nM of His-*Bh*Xyl43 in 50 mM Tris/HCl pH8 supplemented with 1 mg/ml of BSA, at 45 °C for 1 h. (C) HPAEC-PAD analysis of the hydrolysis of A^2XX by His-*Bh*Xyl43. Standard arabinose (red), standard xylooligosaccharides (green) and 1 mM A^2XX incubated with 60 nM of His-*Bh*Xyl43 in 50 mM Tris/HCl pH8 supplemented with 1 mg/ml of BSA, at 45 °C for 24 h (purple).

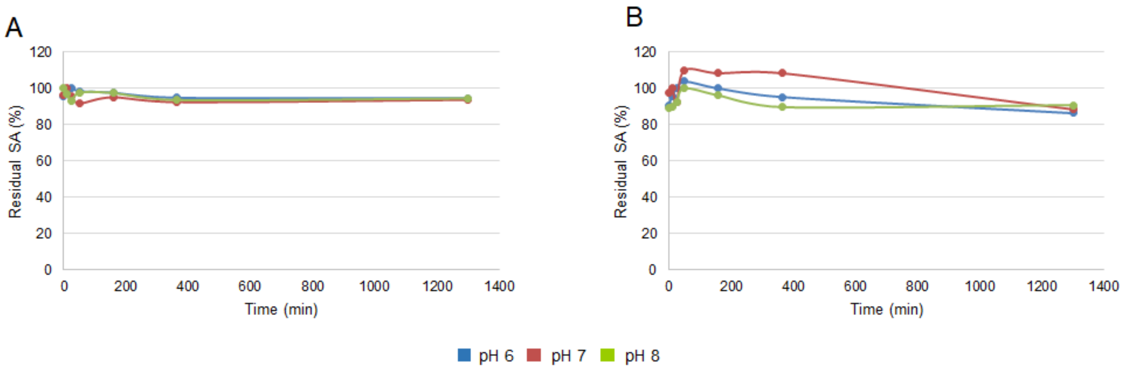

Figure S5. Enzyme stability in 50 mM Phosphate, supplemented with 1 mg/ml of BSA at 37 °C over 24 h, at pH 6, 7 and 8. (A) Residual specific activity of 5 nM of His-*Np*Xyn11A tested on 5 mM *p*NP-X_3. (B) Residual specific activity of 5 nM of His-*Bh*Xyl43 tested on 5 mM *p*NP-X.

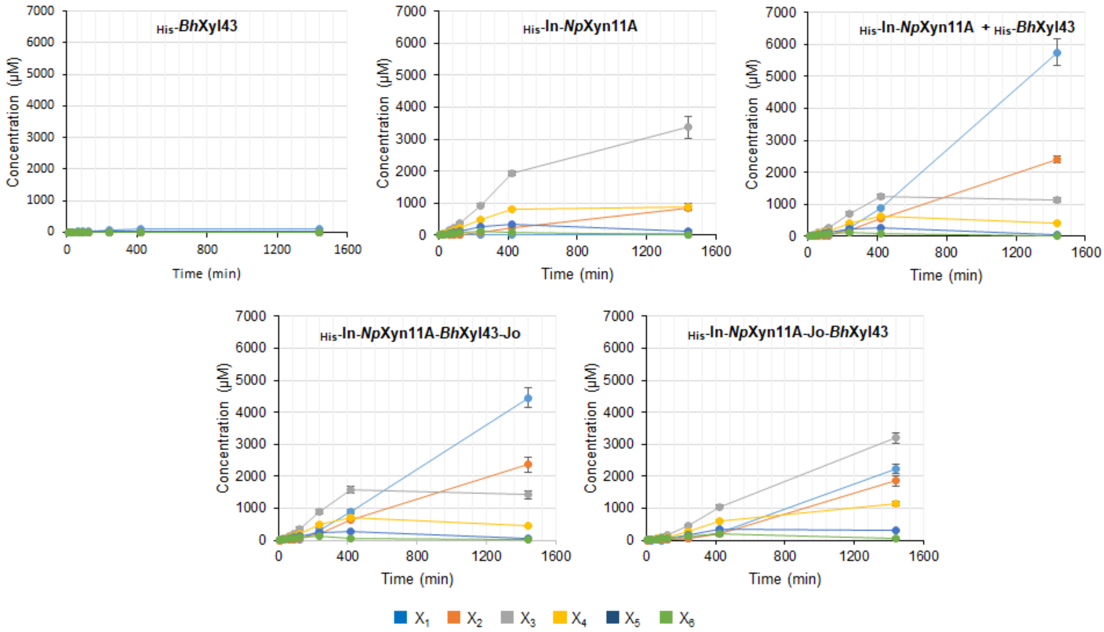

Figure S6. Example of curves of released oligosaccharides from X_1 to X_6 obtained from HPAEC-Pad analysis during 24 h of hydrolysis of 1% Beechwood xylan with various enzymes at pH 7 displaying the standard deviations. The average values were used to plot the graphs on Fig 5. Curved from His-*Bh*Xyl43 are presented as control.

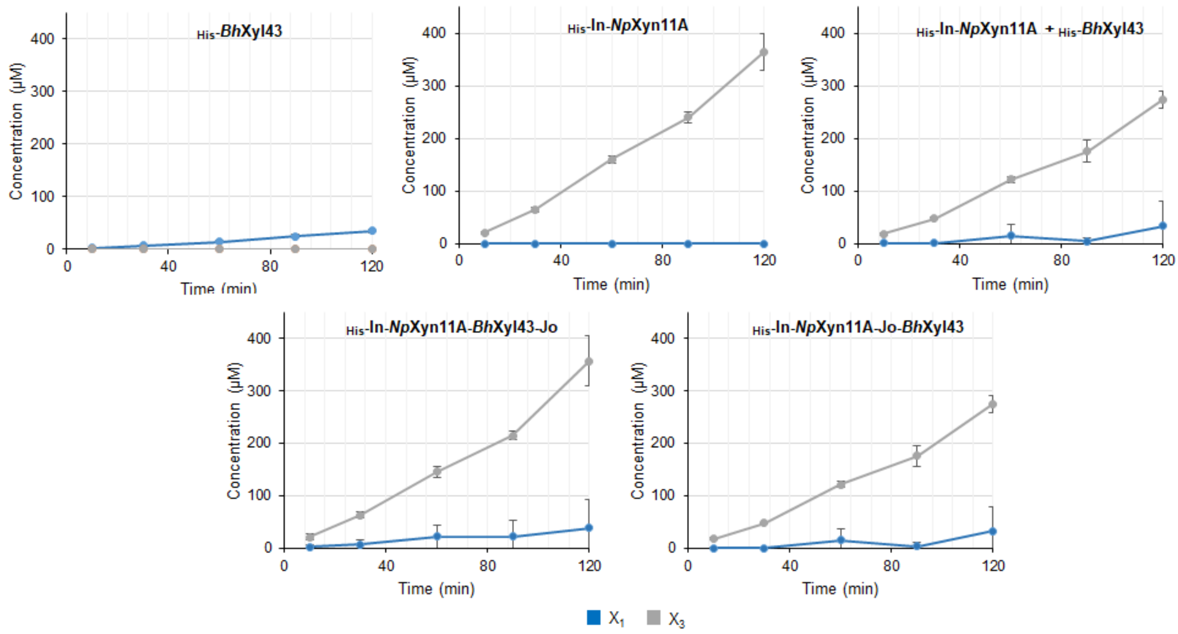

Figure S7. Closer view of the concentration of X1 (blue line) and X3 (gray line) released from 1% Beechwood xylan over the time (between 0 and 120 min). Data extracted from Fig 4 SI. Enzyme concentration set at 1 nM, 10 50 mM phosphate pH 7, 1 mg/ml BSA.

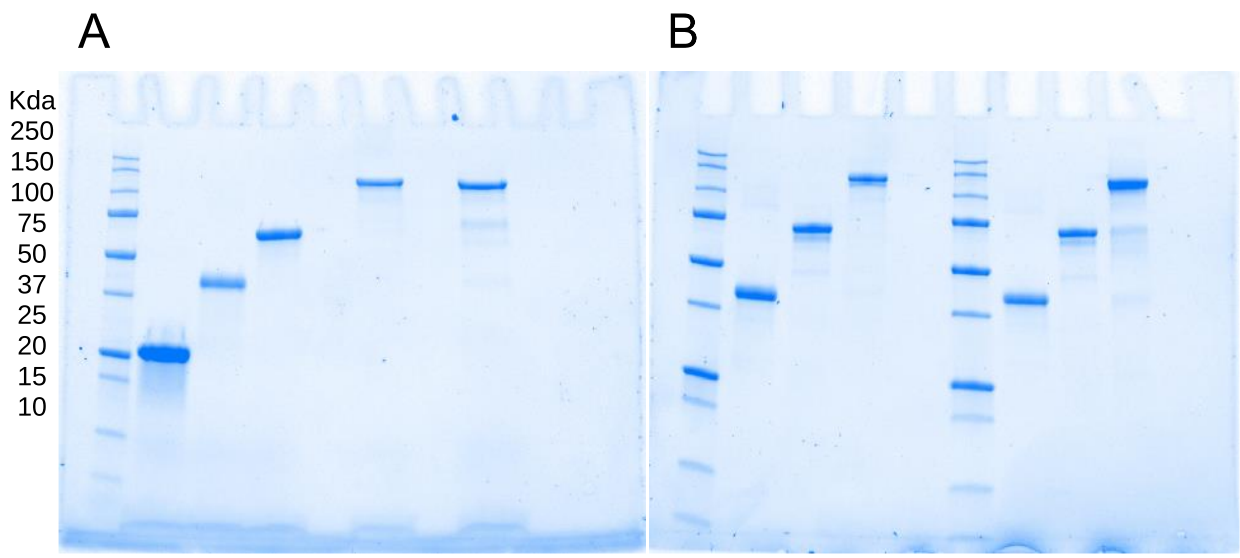

Figure 8. Original SDS-PAGE view. (**A**) Lanes: 1, molecular mass markers; 2, His-*Np*Xyn11A; 3, His-In-*Np*Xyn11A; 4, His-*Bh*Xyl43; 5, empty; 6, His-In-*Np*Xyn11A-Jo-*Bh*Xyl43; 7, empty; 8, His-In-*Np*Xyn11A-*Bh*Xyl43-Jo. (**B**) Lanes: 1, molecular mass markers; 2, His-In-*Np*Xyn11A; 3, His-Jo-*Bh*Xyl43; 4, His-In-*Np*Xyn11A-Jo-*Bh*Xyl43; 5, empty; 6, molecular mass markers; 7, His-In-*Np*Xyn11A; 8, His-Jo-*Bh*Xyl43; 9, His-In-*Np*Xyn11A-*Bh*Xyl43-Jo.

2. Tables S1 to S3

Table S1. : Biophysical parameters of $_{His}$-In-*Np*Xyn11A-Jo-*Bh*Xyl43 and $_{His}$-In-*Np*Xyn11A-*Bh*Xyl43-Jo complexes calculated from both SAXS curves shown in Figure S2 with ATSAS suite programs [2].

	R_g (Å)	D_{max} (Å)	Porod's volume (Å^3)	Calculated molecular weight (kDa)	Theorical molecular weight (kDa)
$_{His}$-*Bh*Xyl43	38.2	136	64,300	128.4	121.4
$_{His}$-In-*Np*Xyn11A-Jo-*Bh*Xyl43	56.4	230	278,000	189.1	223.9
$_{His}$-In-*Np*Xyn11A-*Bh*Xyl43-Jo	50.5	200	282,000	180.5	223.7

Table S2. Molar extinction coefficient and molecular weight of the enzymes studied in this work.

Enzyme	Molar extinction coefficient ($M^{-1}.cm^{-1}$)	Molecular weight (g/mol)
*Np*Xyn11A-$_{His}$	61,880	25,981
$_{His}$-In-*Np*Xyn11A	69,330	41,544
$_{His}$-*Bh*Xyl43	119,095	11,9095
$_{His}$-Jo-*Bh*Xyl43	129,525	72,164
Jo-*Bh*Xyl43	129,525	70,470
*Bh*Xyl43-Jo	129,525	70,339
$_{His}$-In-*Np*Xyn-Jo-*Bh*Xyl43	198,855	111,997
$_{His}$-In-*Np*Xyn-*Bh*Xyl43-Jo	198,855	111,886

Table S3. Synthetic gene sequences.

Jo-BhXyl43	***BhXyl43-Jo***
CCATGGGCGCTAGCCAGGATCCGTCTGACC	CCATGGGCGCTAGCGTCAATCGTATCCAAAAT
AGTATCCACAAACAGGGACTTATCCAGATG	CCTATTTTGCCAGGGTTTCATCCAGACCCATCC
TTCAAACACCTTATCAGATTATTAAGGTAG	ATTGTCCGTGTTGGTGATGATTACTATATCGCC
ATGGTTCGGAAAAAAACGGACAGCACAAG	ACCTCTACATTTGAATGGTTTCCTGGGGTGCGC
GCGTTGAATCCGAATCCATATGAACGTGTG	ATCCACCATTCTCGGGATTTAAAACATTGGCG
ATTCCAGAAGGTACACTTTCAAAGAGAATT	CTTTGTATCTAGTCCGCTGACCCGCACTTCCCA
TATCAAGTGAATAATTTGGATGATAACCAA	ACTAGACATGAAAGGGAATATGAACTCCGGCG
TATGGAATCGAATTGACGGTTAGTGGGAAA	GGATATGGGCGCCATGCCTAAGCTATCATGAC
ACAGTGTATGAACAAAAAGATAACGTCGAC	GGAACCTTTTATTTGATCTATACTGATGTGAAG
ATGGTCAATCGTATCCAAAATCCTATTTTGC	CAATGGCACGGTGCCTTCAAAGACGCGCACAA
CAGGGTTTCATCCAGACCCATCCATTGTCC	CTATTTAGTGACGGCACAAAACATTGAAGGGC
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TACATTTGAATGGTTTCCTGGGGTGCGCATC	GGCTTTGACCCGTCCCTGTTTCACGATGACGAT
CACCATTCTCGGGATTTAAAACATTGGCGC	GGCCGAAAATGGCTCGTTAACATGATCTGGGA
TTTGTATCTAGTCCGCTGACCCGCACTTCCC	CTACCGCAAAGGAAACCATCCTTTTGCCGGAA
AACTAGACATGAAAGGGAATATGAACTCCG	TTATTTTGCAAGAATACTCAGAAGCAGAACAA
GCGGGATATGGGCGCCATGCCTAAGCTATC	AAACTTGTCGGGCCTGTGAAAAATATCTATAA
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CGCGCACAACTATTTAGTGACGGCACAAAA
CATTGAAGGGCCGTGGTCGGACCCGATTTA
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GTTTCACGATGACGATGGCCGAAAATGGCT
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TTGCCGAAGGAGGGACGGAATACGAACAC
GCCGCGACCCTCGCCCGCTCACAGTCAATT
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CCACTCGTCACATCGACTGGCCAGCCGGAA
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CCGAGCTCGATGATTTTGACGCACCCCAGC
TGCACCATCAATGGAACACGCTGCGCATCC
CTGCCGACCCATCATGGTGCTCGCTCGAGG
AACGTCCGGGCCATTTACGACTGCGCGGGA
TGGAGTCCCTCACTTCCGTCCACTCGCAAA
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AACAAAAAGATAACGTCGACTAACAAGCTT

Reference

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2. Konarev, P.V.; Volkov, V.V.; Sokolova, A.V.; Koch, M.H.J.; Svergun, D.I. PRIMUS : A Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* **2003**, *36*, 1277–1282, doi:10.1107/S0021889803012779.

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