Arabinohydrolases from *Chrysosporium lucknowense* and their use to degrade sugar-beet pulp

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Dr. Yvonne Westphal Dr. Henk Schols Prof. Dr. Harry Gruppen

Wageningen University - Lab of Food Chemistry

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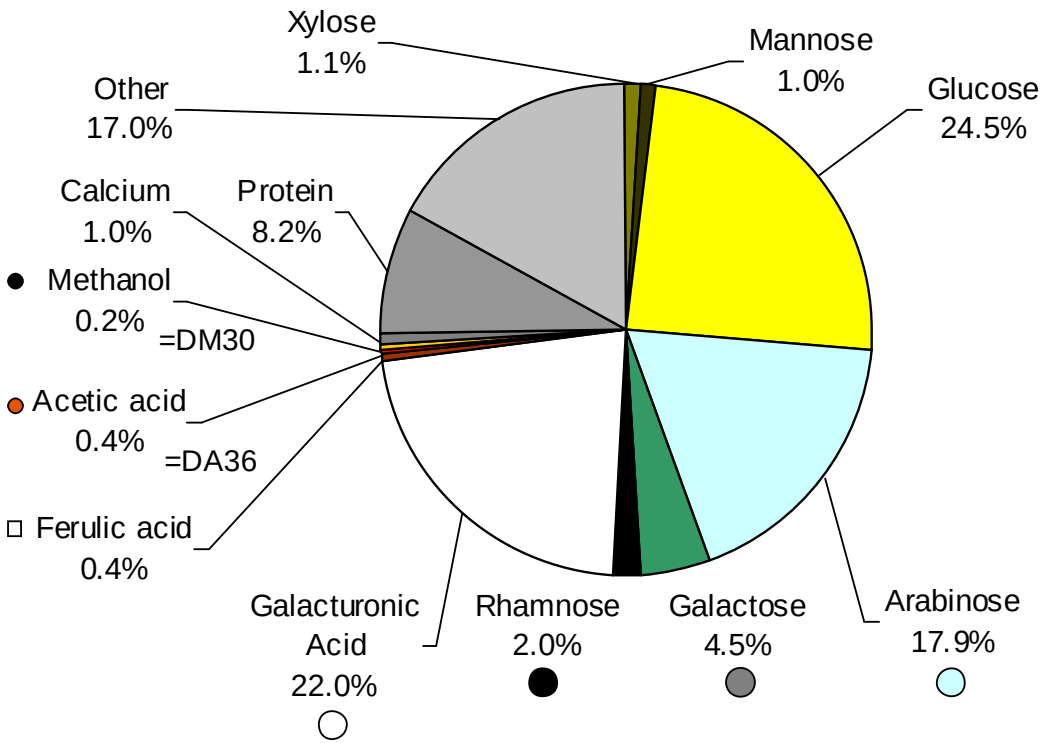
The cell wall of sugar beet pulp



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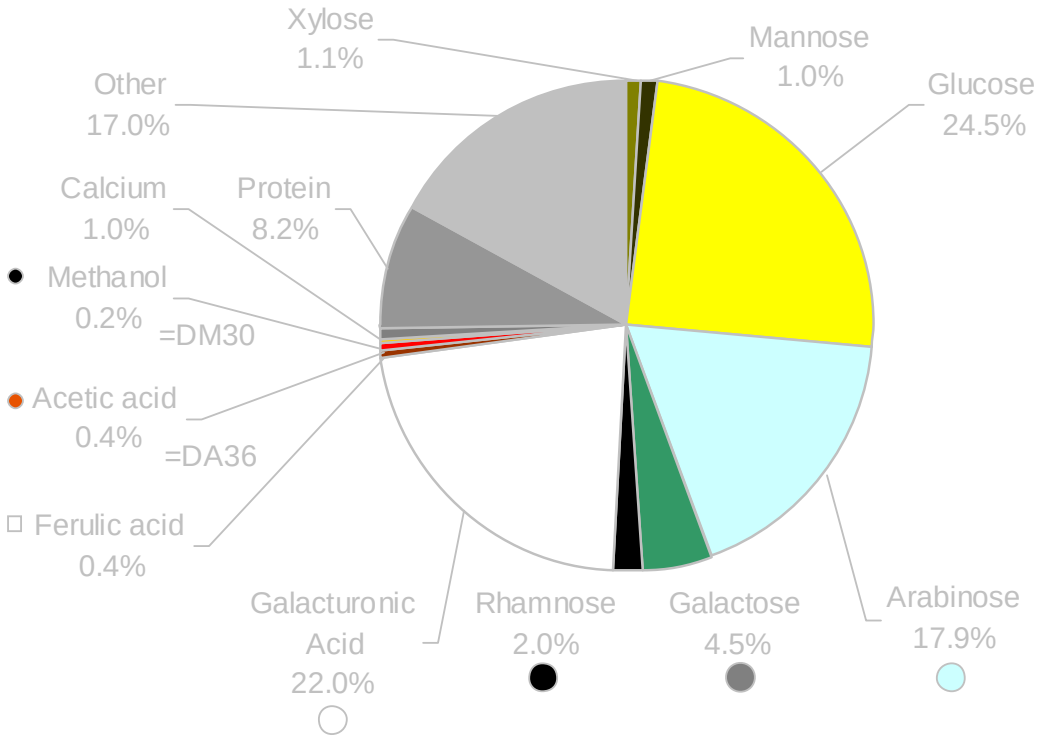

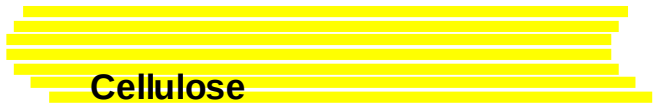

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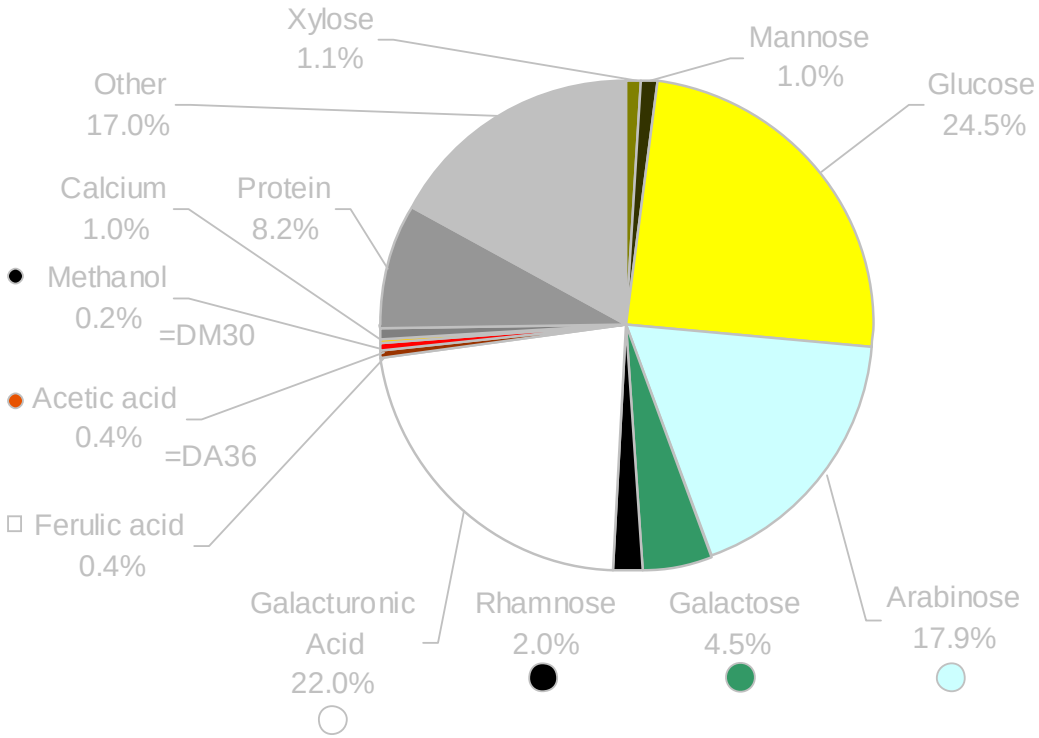

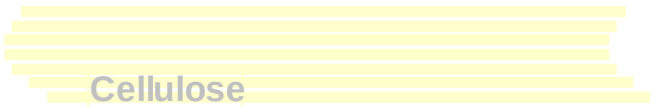

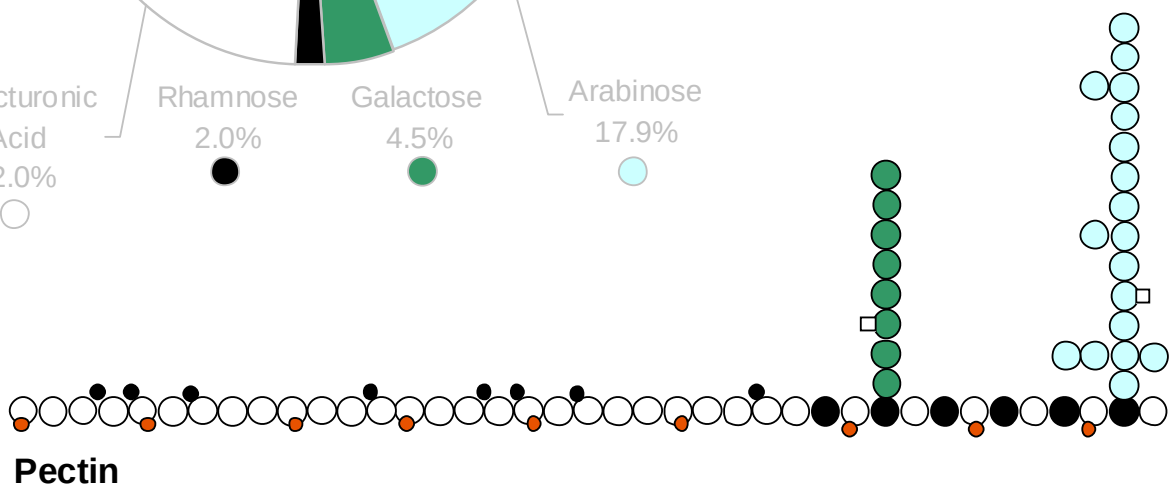

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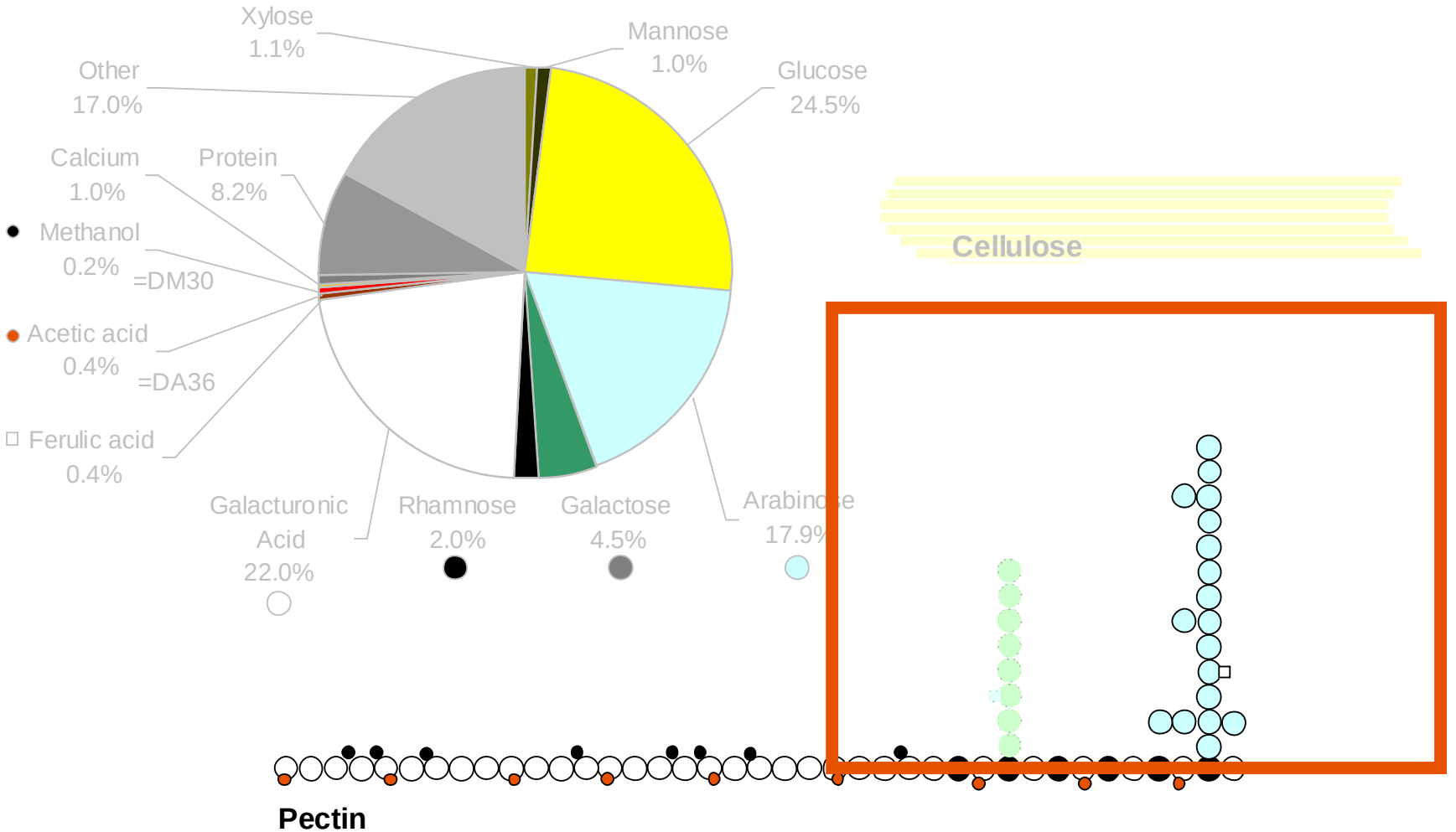

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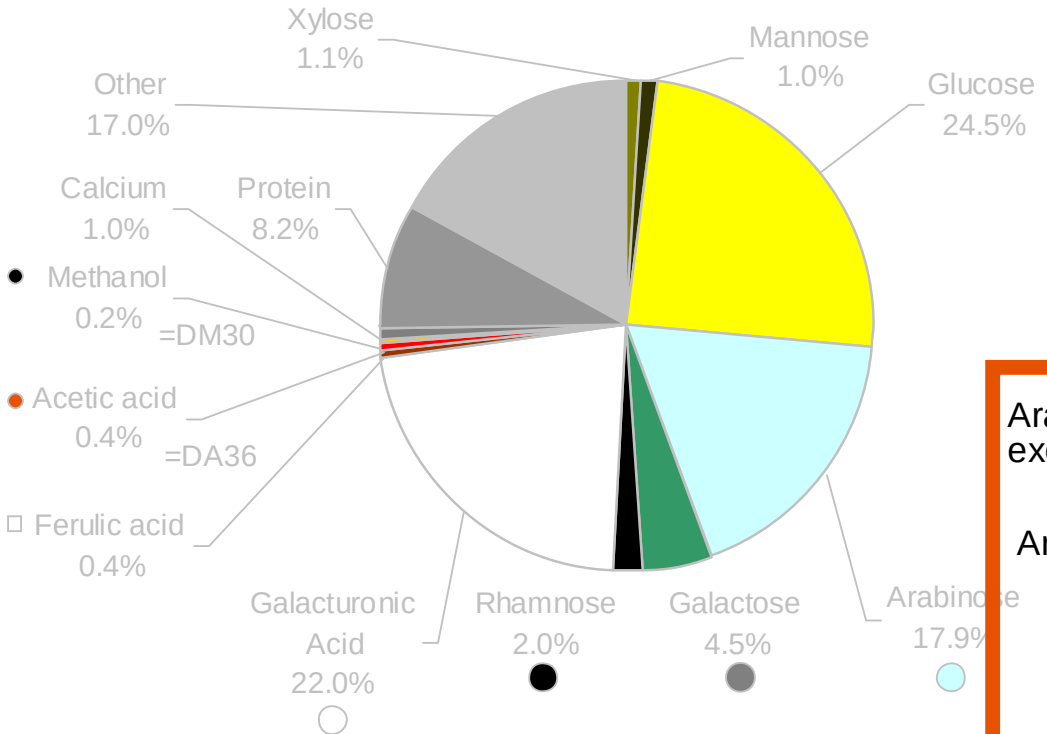

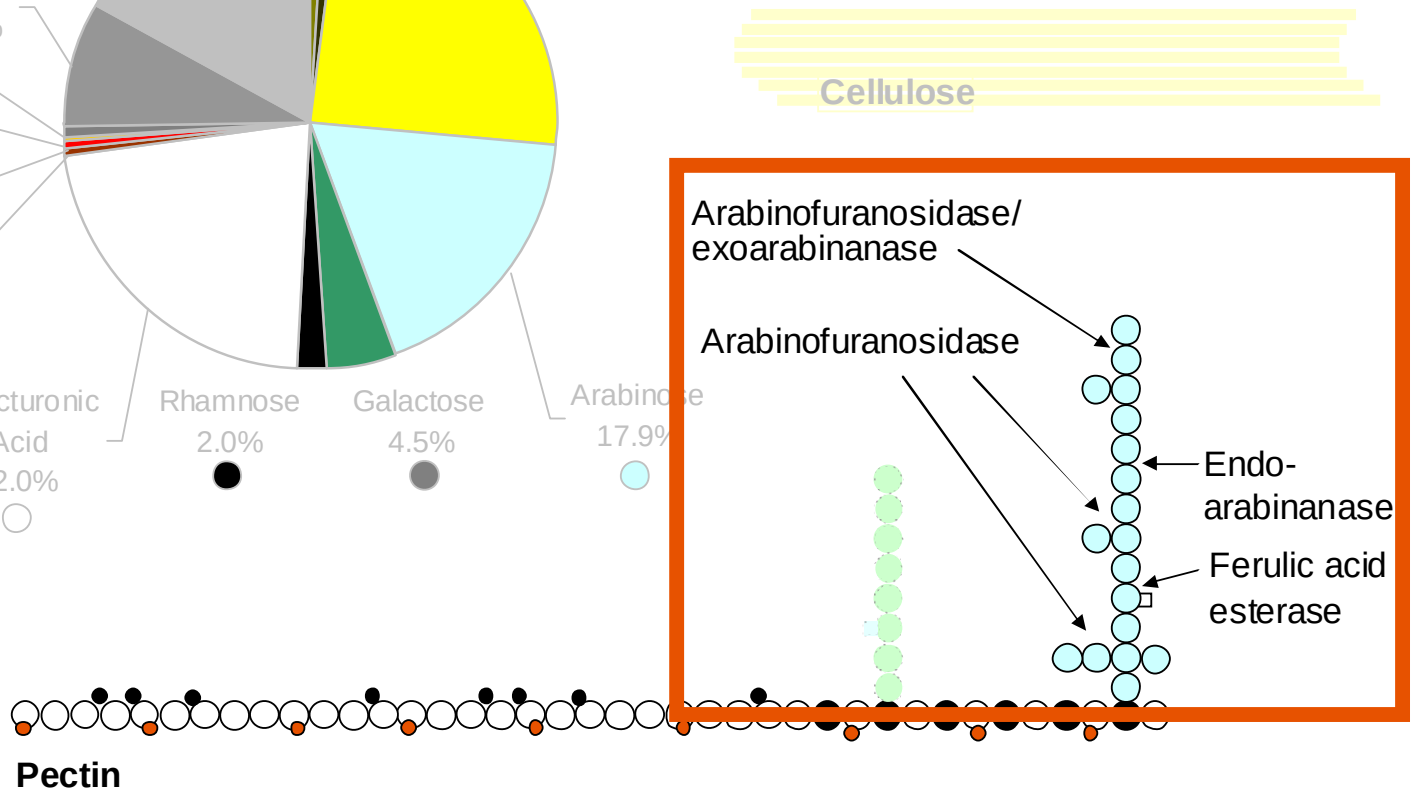

The enzymatic toolbox of C1

Annotated enzyme	Number of enzymes in C1	*T. reesei**	*A. niger***
β-galactosidases	5	2	6
β-glucosidases / β-xylosidases	11	12	17
Endo-glucanases / galactanases	8	8	10
Polygalacturonases	2	4	21
Arabinanases / Arabinofuranosidases / β-xylosidases	10	2	10
Arabinofuranosidases	2	-	2
Galactanases	1	-	2
α-rhamnosidases	1	-	8
Exo-arabinanases	2	-	-
Ferulic acid esterases	6	-	3
Rhamnogalacturonan acetyl esterases	2	-	2
Pectin methyl esterases	1	-	3
Lyases	7	-	8
Total	**58**	**28**	**92**

*from the GJI database, **from the CAZy database

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Biochemical properties of C1 arabinohydrolases

	Abn1	Abn2	Abn4	Abf3
GH family	43	93	43	51
Mode of action	endo	exo	exo	exo
pH optimum	5.5	4.5	5.5	5.0
pH stability	5-8	6-7	6-8	5-7
Temp. optimum (°C)	55	50	55	40
Spec. Activity (U/mg)	26.0	7.1	9.5	21.4
Substrate	linear arabinan	linear arabinan	branched arabinan	pNP-Ara
Released product	ara+ara2	ara2	ara	ara

Kühnel et al., 2010; Bioresource Technology

Branched arabinose oligomers produced by C1 arabinohydrolases

Component	Schematic structure
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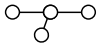
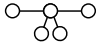
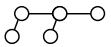
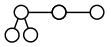
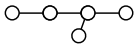
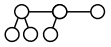
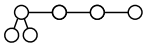
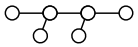
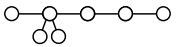
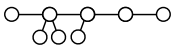
4.1	
4.2	
5.1	
5.2	
5.3	
5.4	
6.1a	
6.1b	
6.2	
7.2	
8.1	

Westphal et al., 2010; Carbohydrate Research

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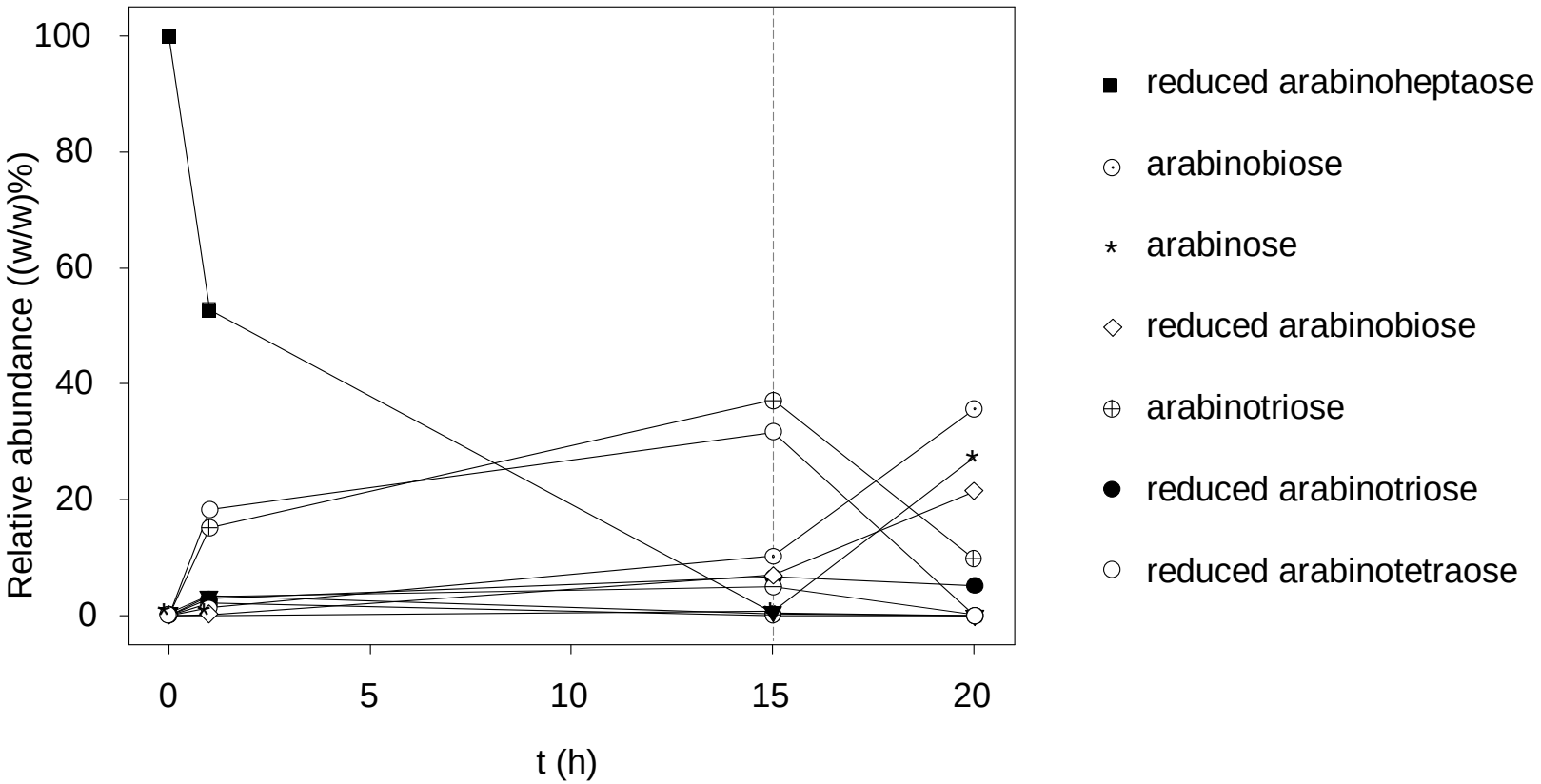
Activity toward reduced linear arabinose oligomers

Degradation of reduced arabinoheptaose by Abn1



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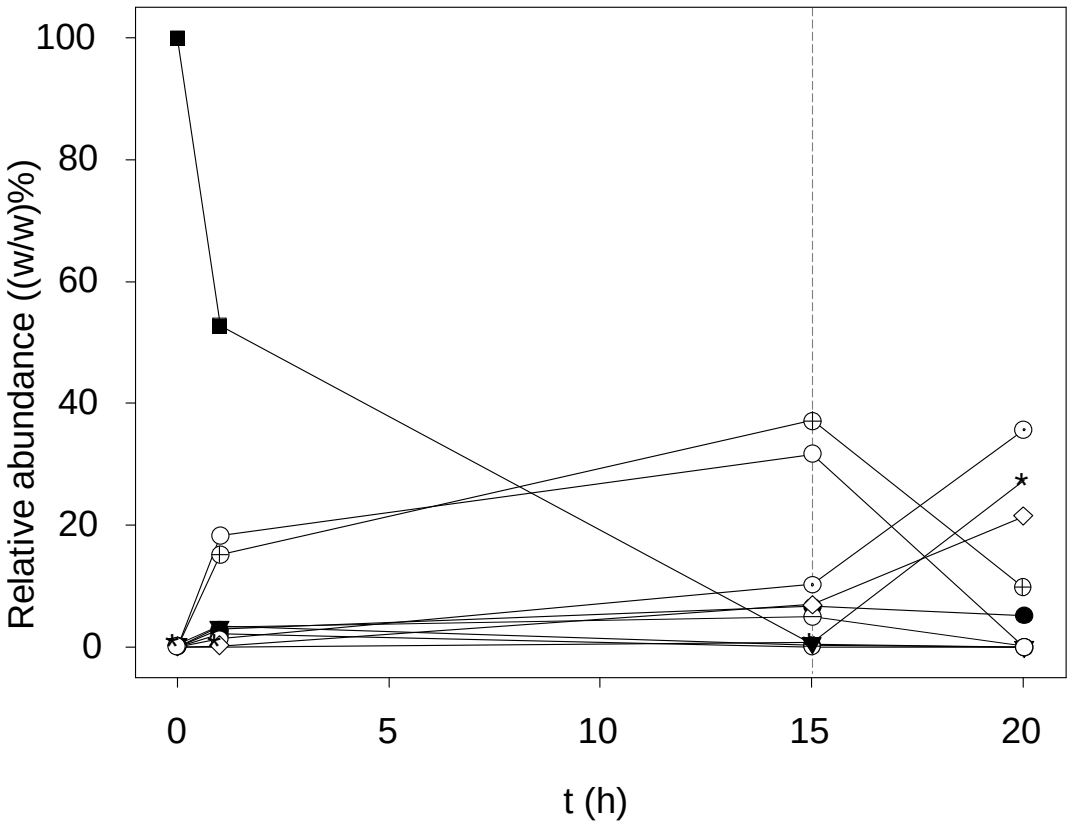
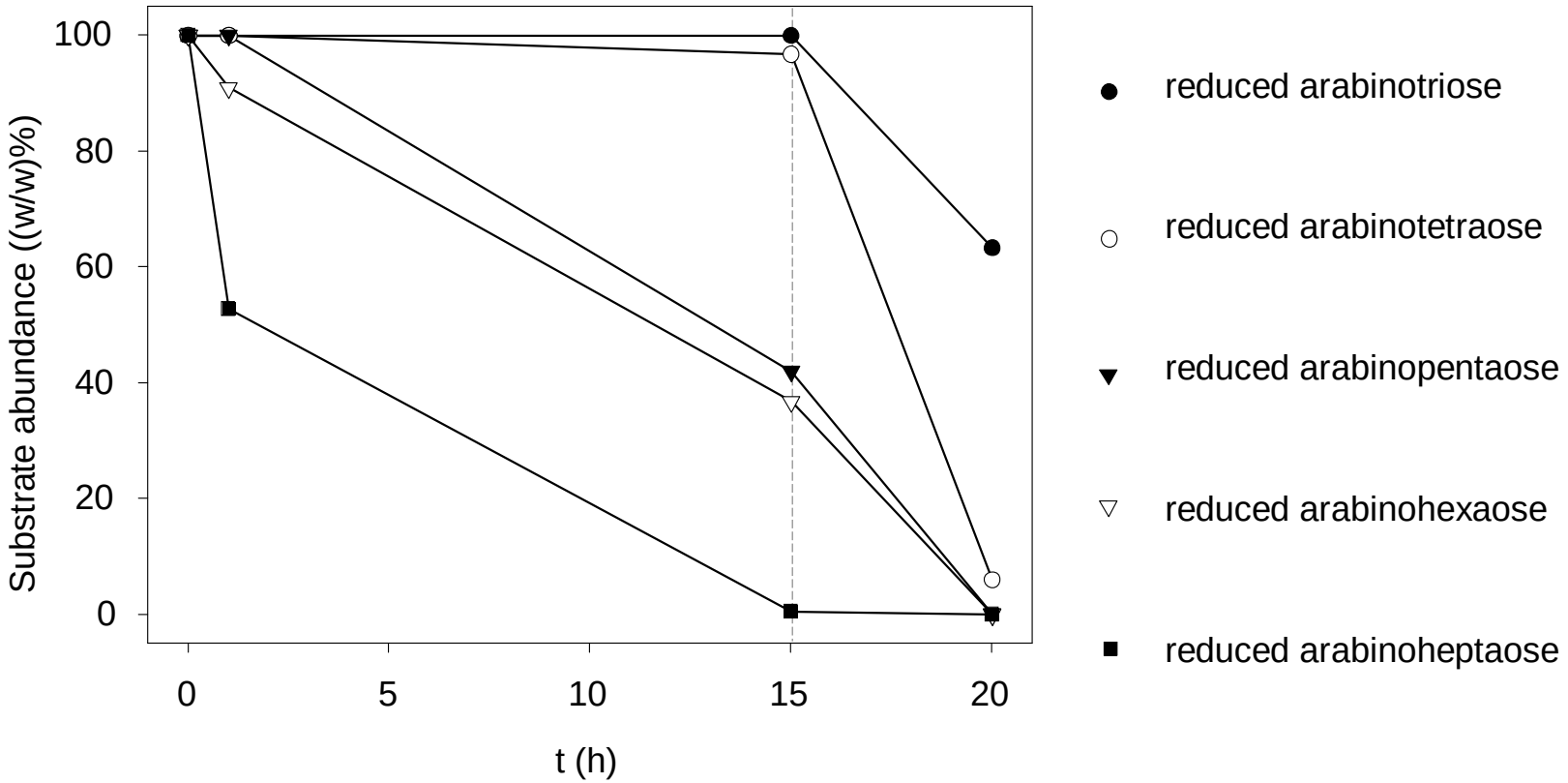


- reduced arabinoheptaose
- arabinobiose
- arabinose
- reduced arabinobiose
- arabinotriose
- reduced arabinotriose
- reduced arabinotetraose

Abn1 activity increases with increasing DP of the substrates

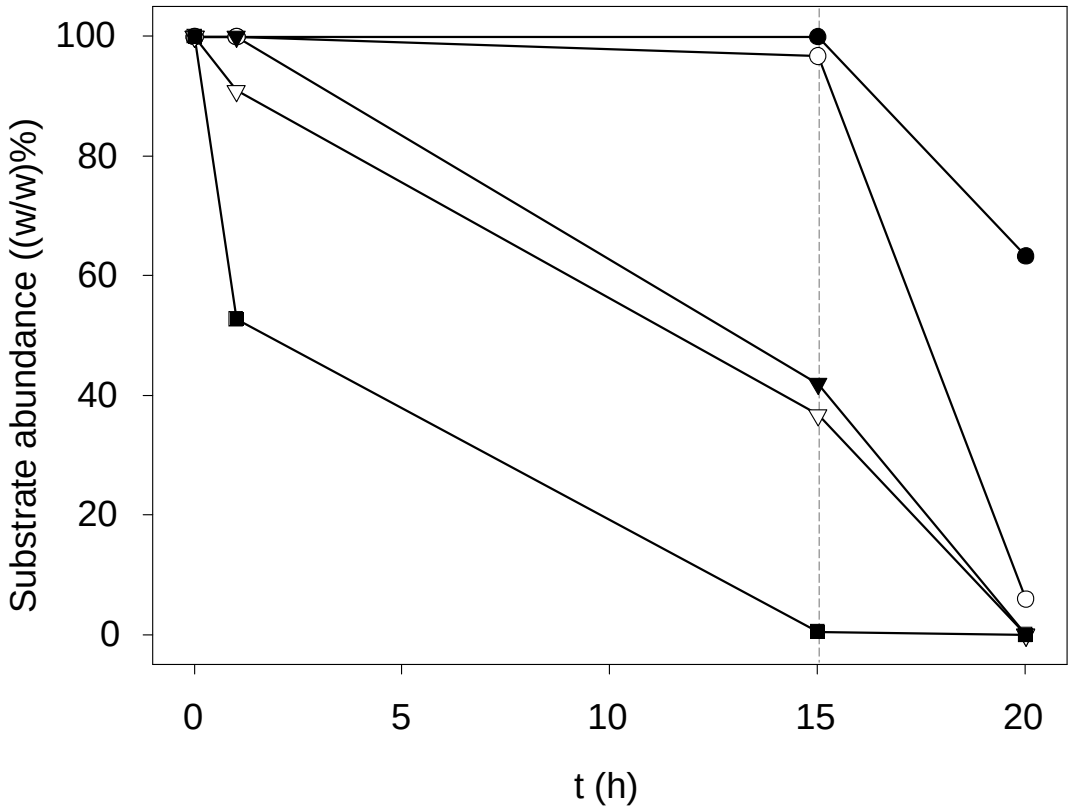
Activity toward reduced linear arabinose oligomers

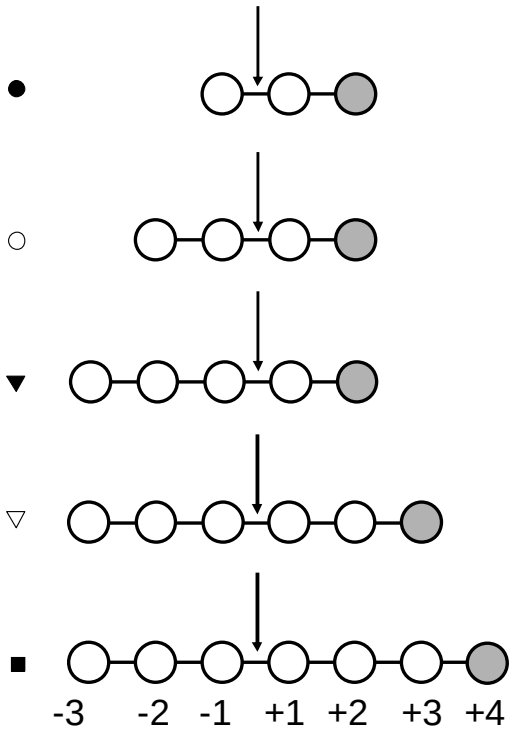
Abn1 toward reduced arabinose oligomers



Activity toward reduced linear arabinose oligomers

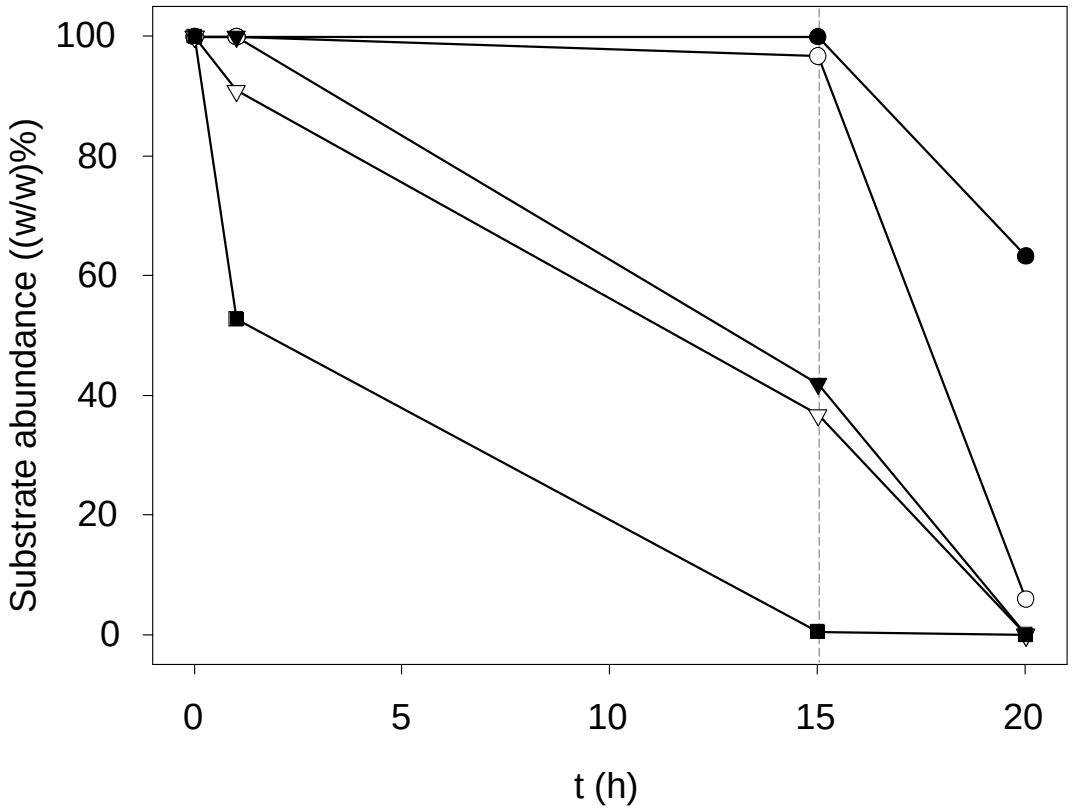
Abn1 toward reduced arabinose oligomers

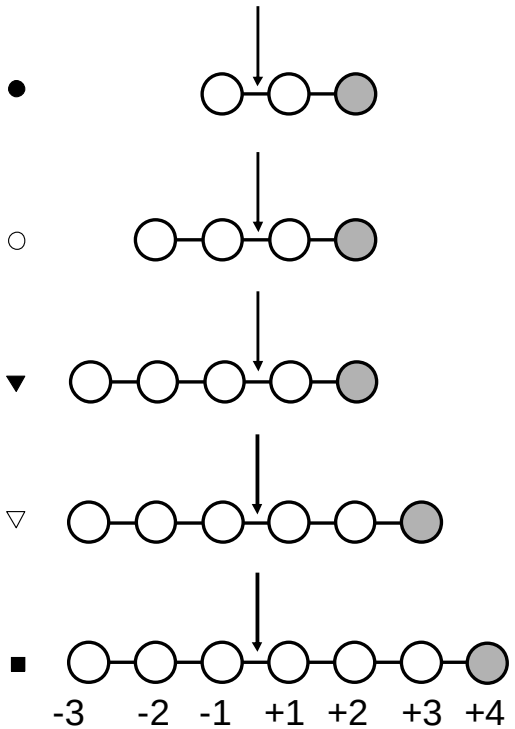




Activity toward reduced linear arabinose oligomers

Abn1 toward reduced arabinose oligomers

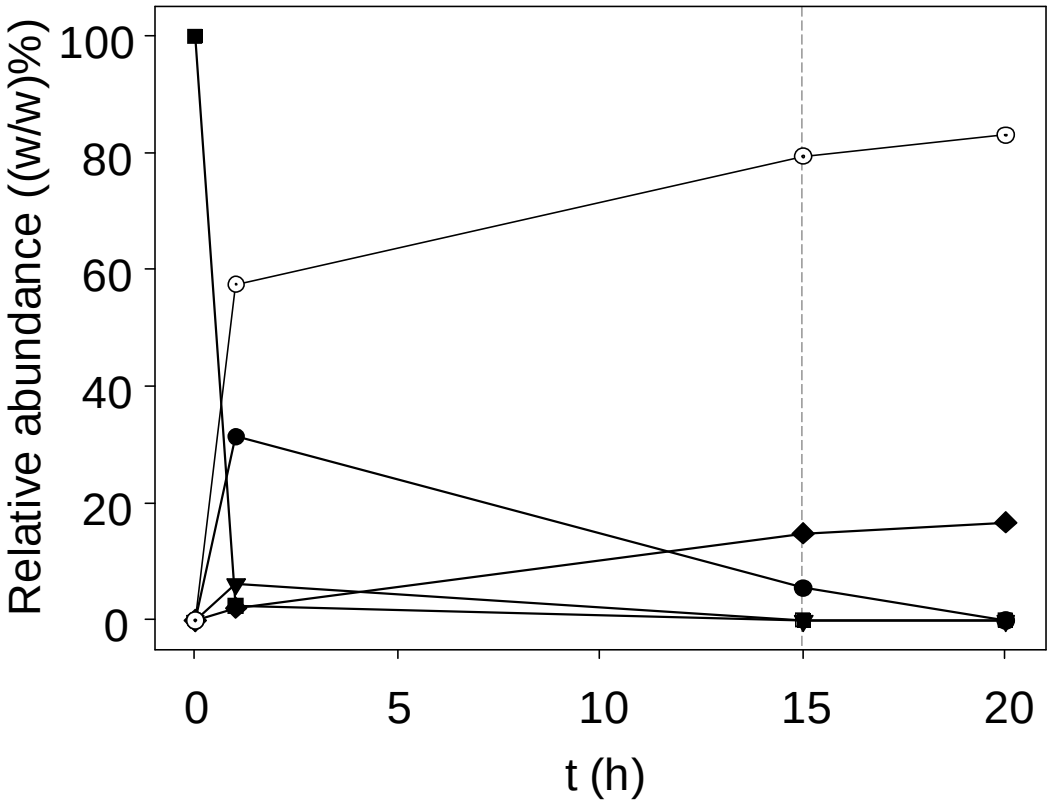


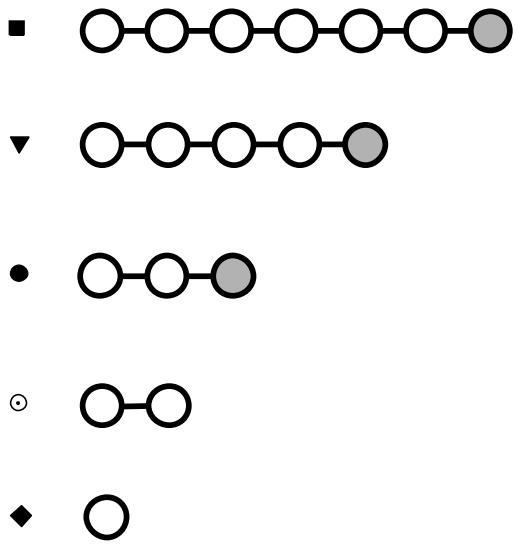


Abn1 has a lower binding capacity at the -2 subsite

Activity toward reduced linear arabinose oligomers

Abn2 releases arabinobiose from the non-reducing end





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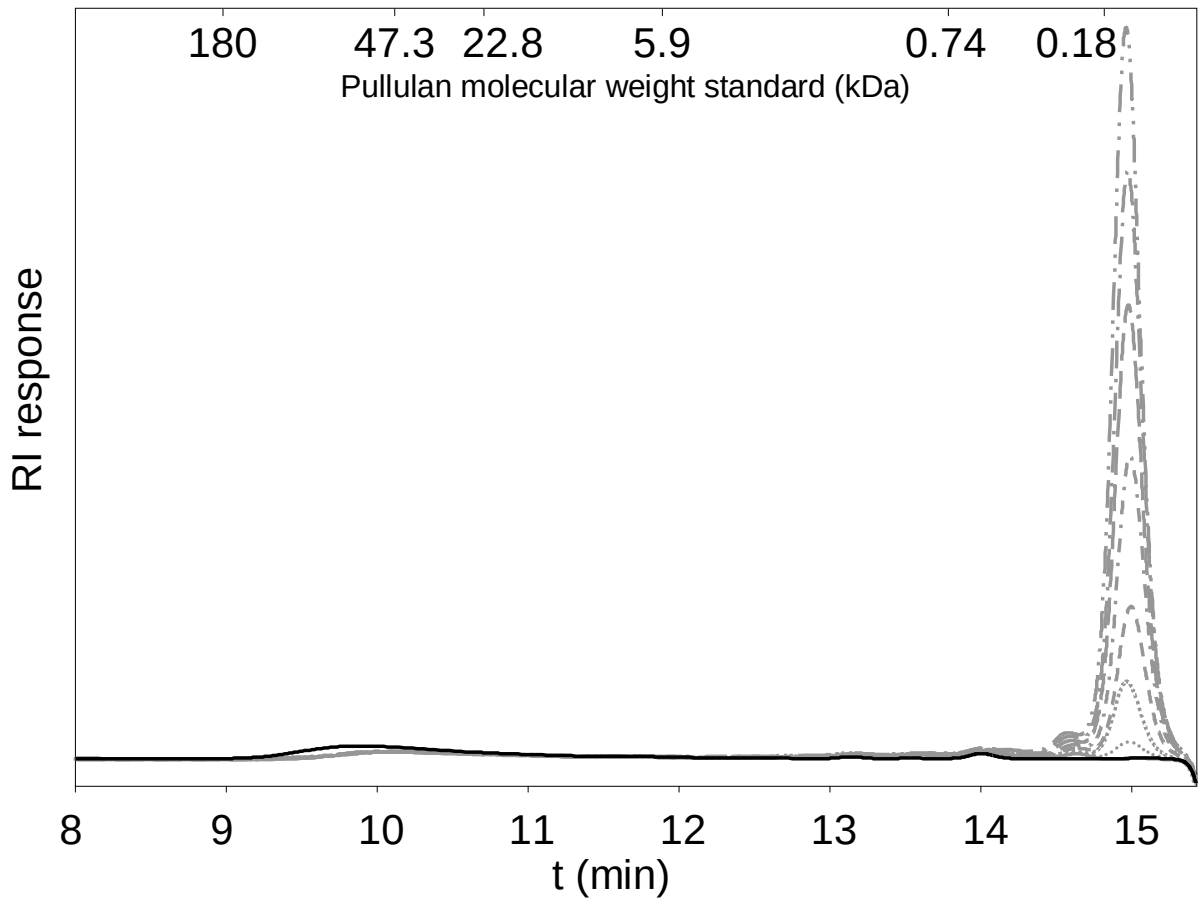
Abn1 is not product inhibited by arabinobiose



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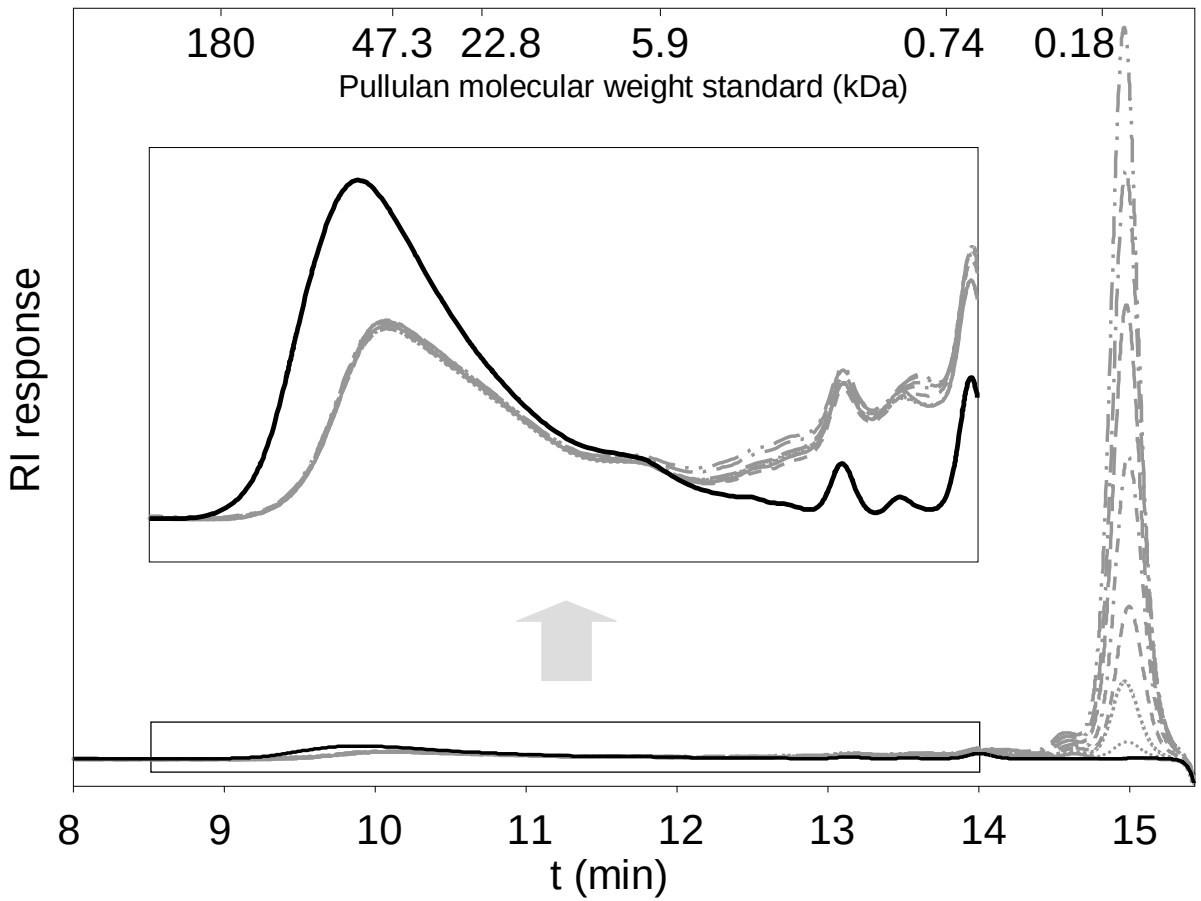
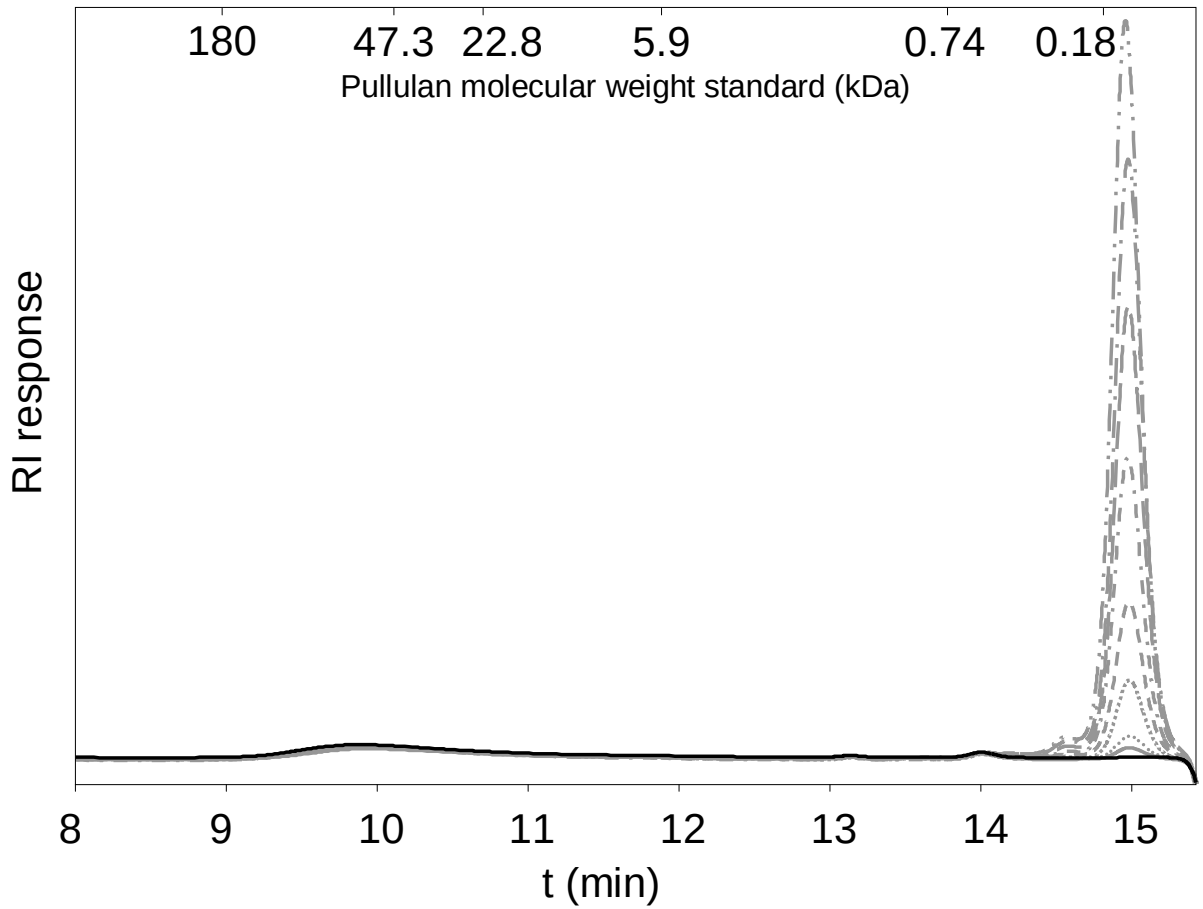


Abn2 is strongly inhibited by arabinobiose



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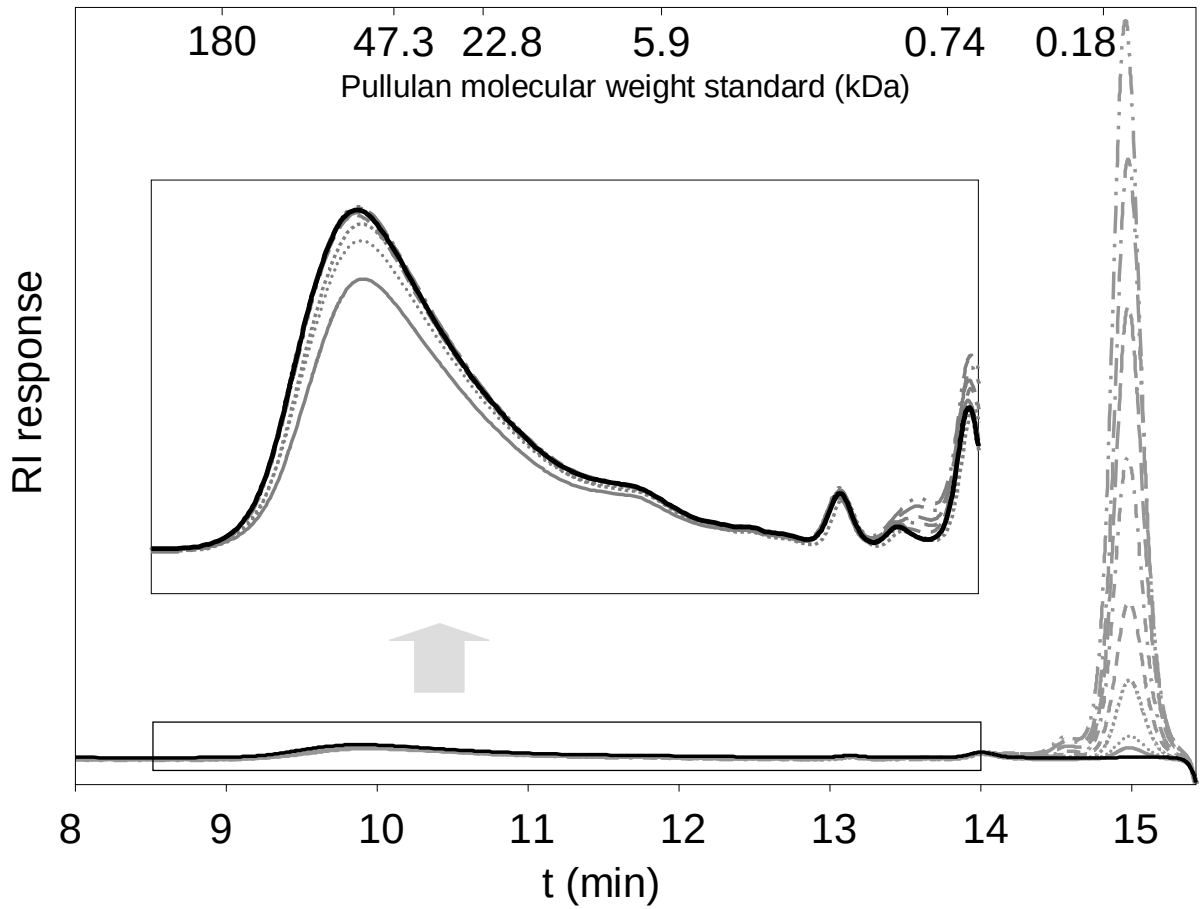
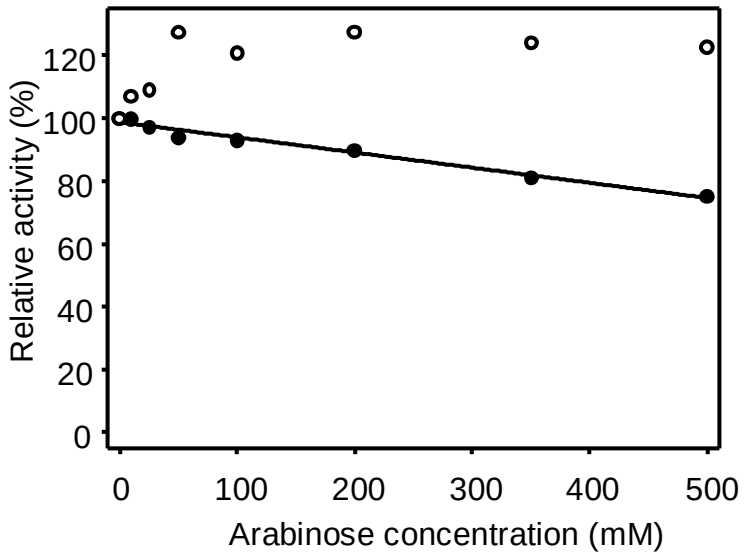


Product inhibition

Abn4 and Abf3



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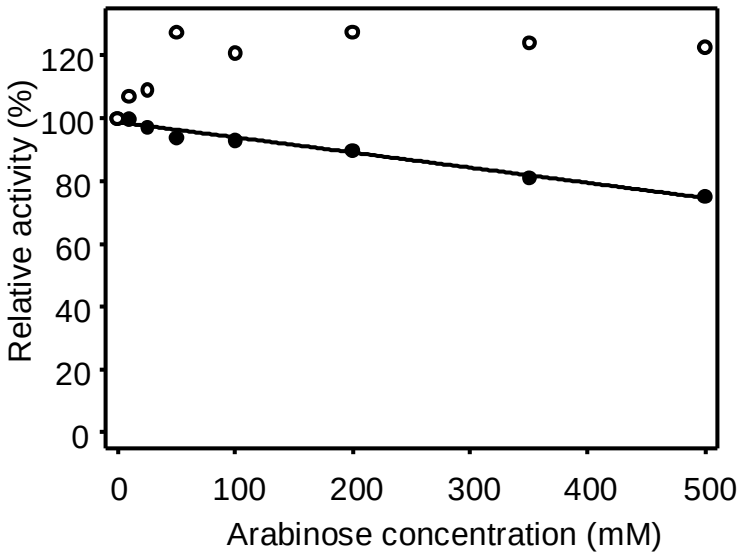

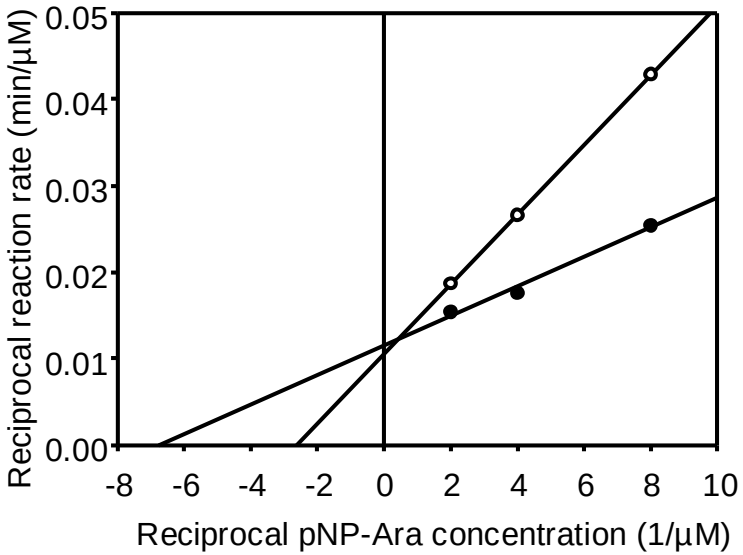

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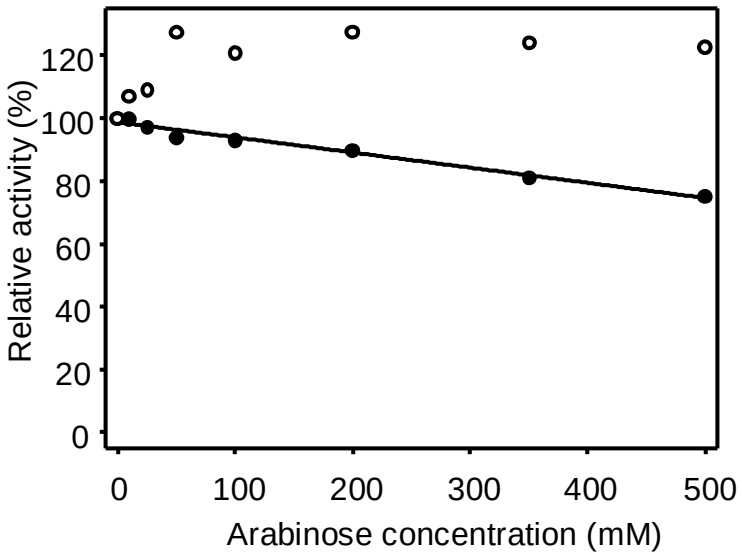

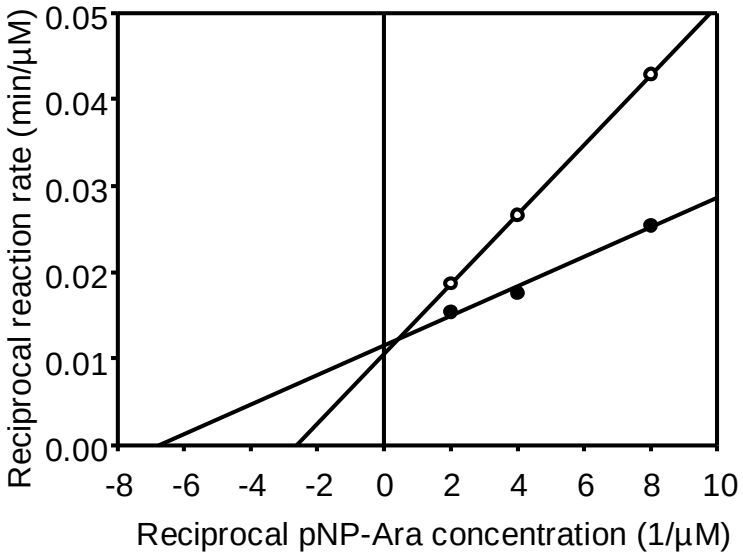

Abf3 shows competitive product inhibition, whereas Abn4 is stabilized by low concentrations of arabinose

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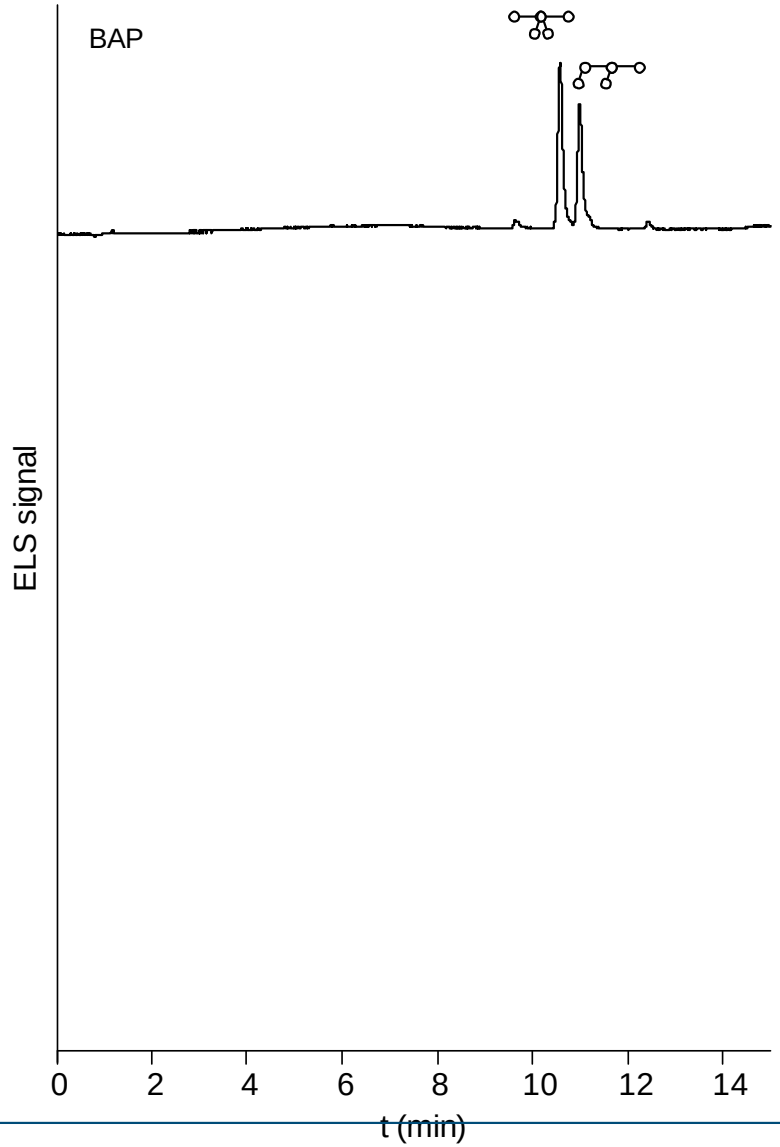
Activity toward branched arabinose oligomers



- Branched arabinopentaose mix includes two isoforms
- Abn4 can only degrade the single substituted isoform
- Abf3 also degrades the single substituted isoform and removes the terminal non-reducing arabinose from the double substitued isoform
- It also removes the double substitution, however, with less activity

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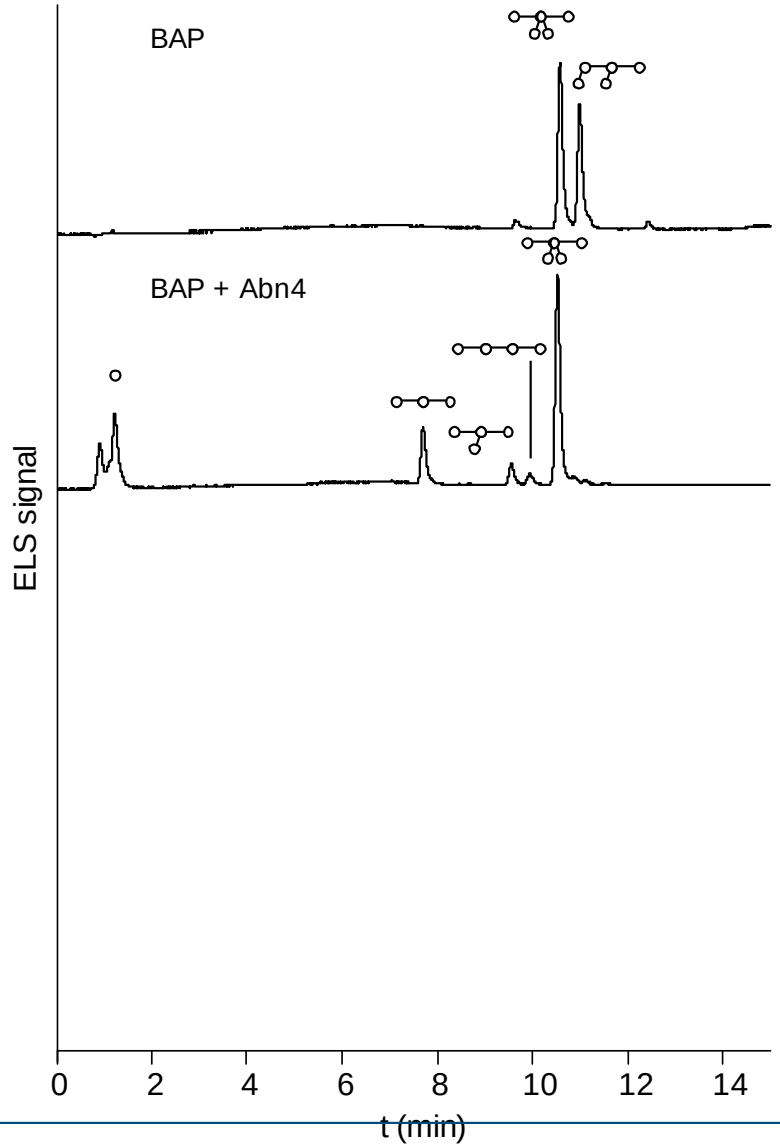

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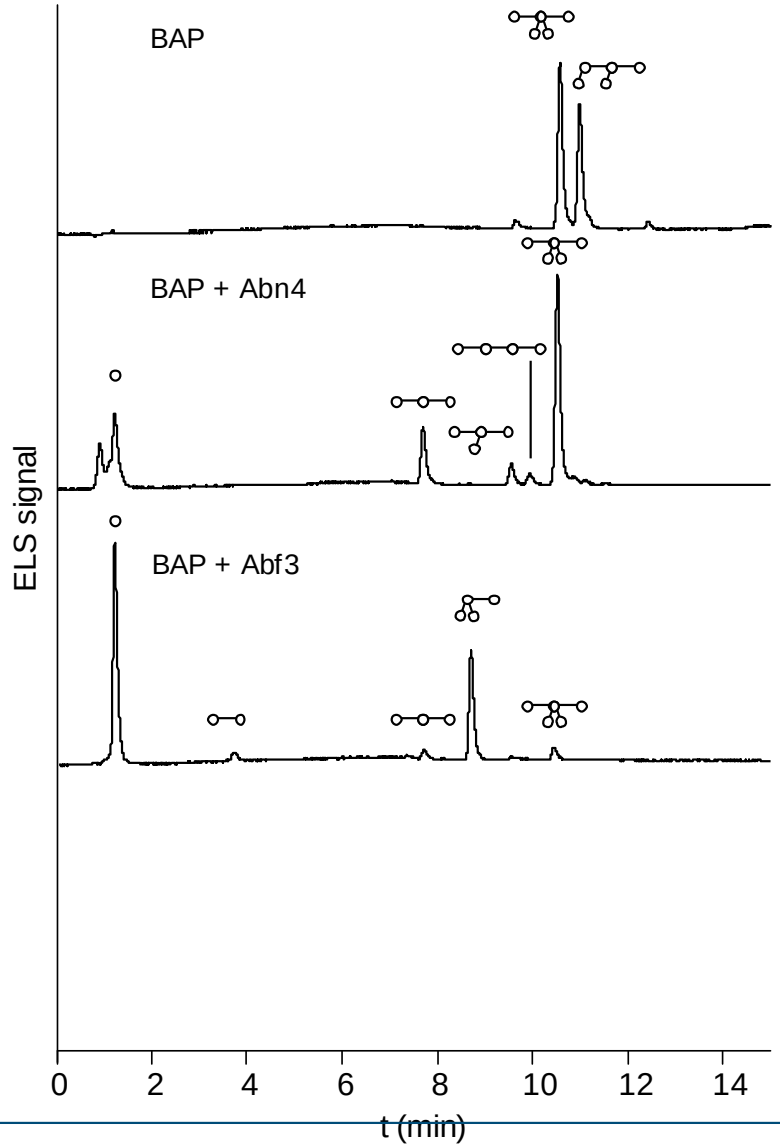

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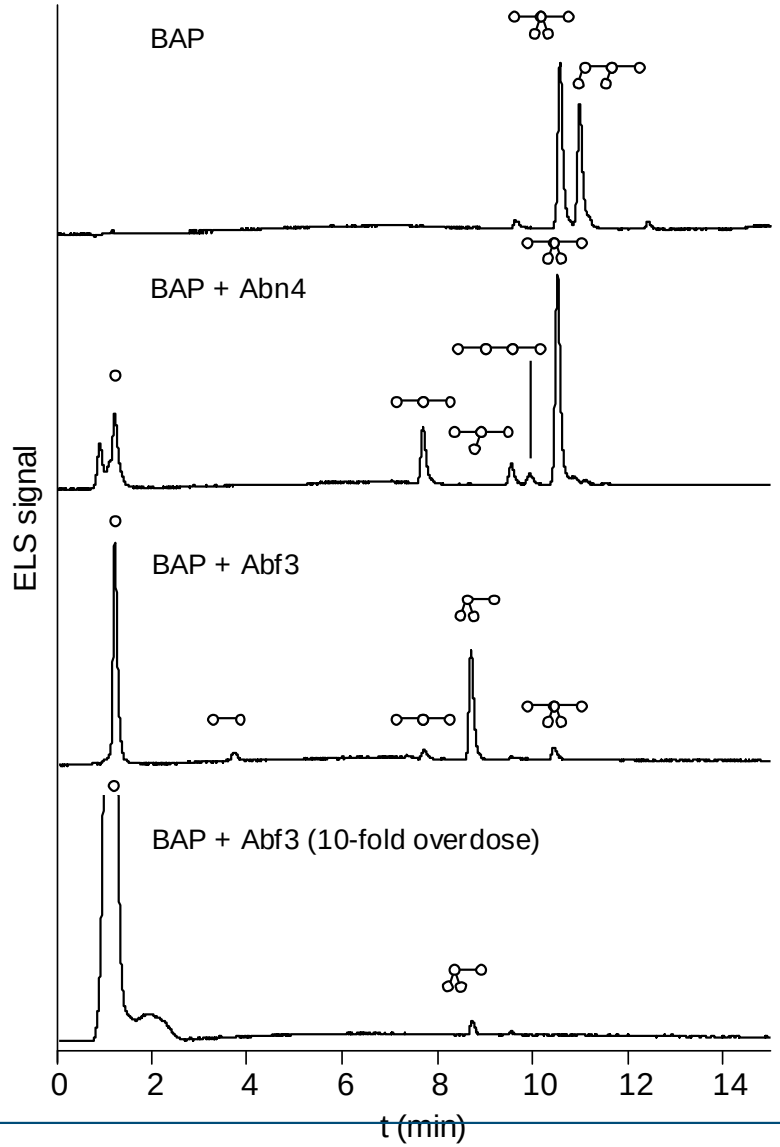

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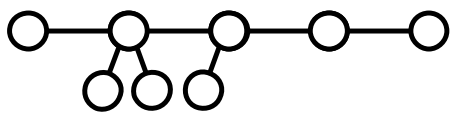
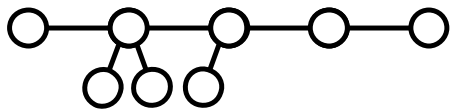
8.1



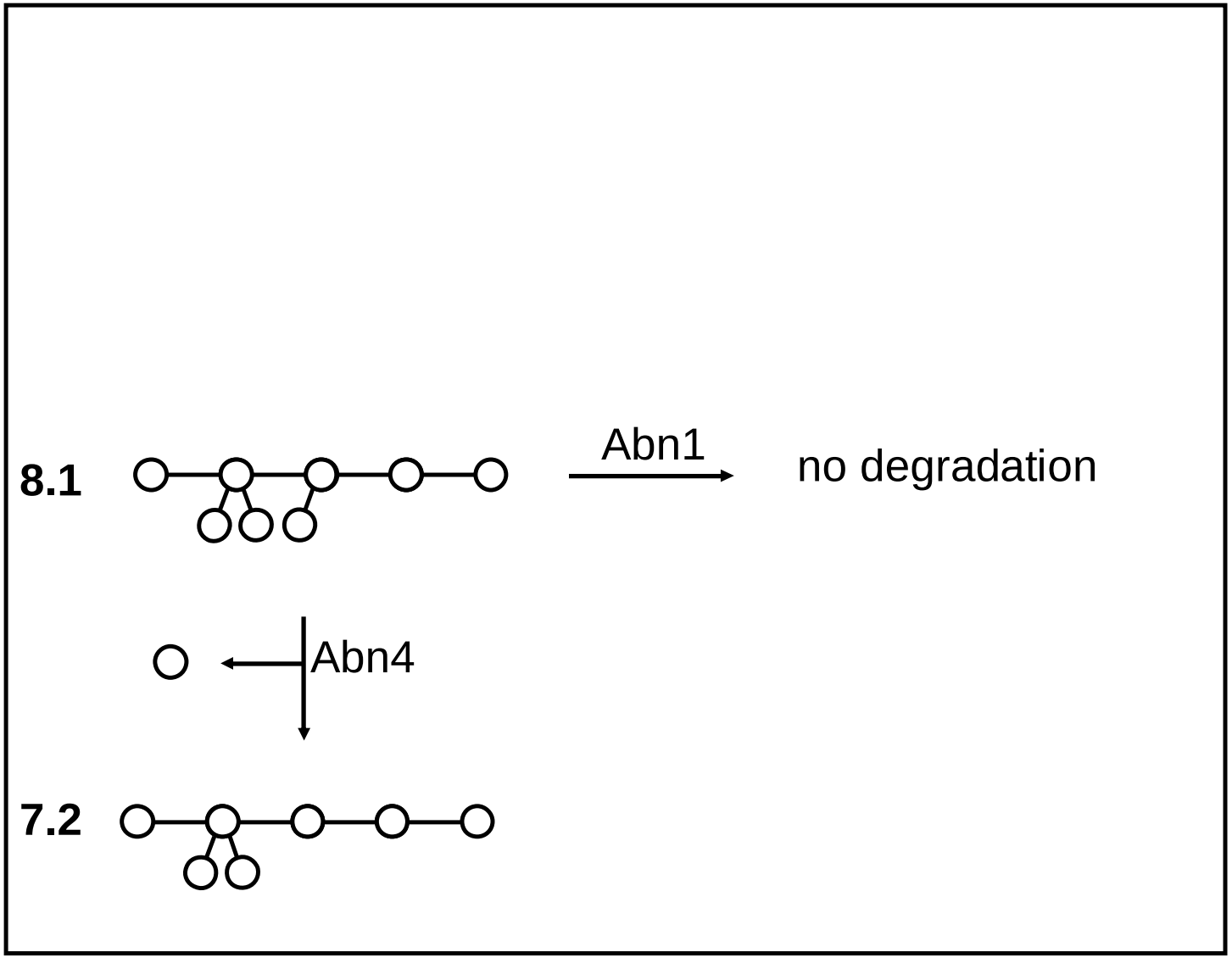
Activity toward branched arabinose oligomers

8.1



Abn1 → no degradation

Activity toward branched arabinose oligomers



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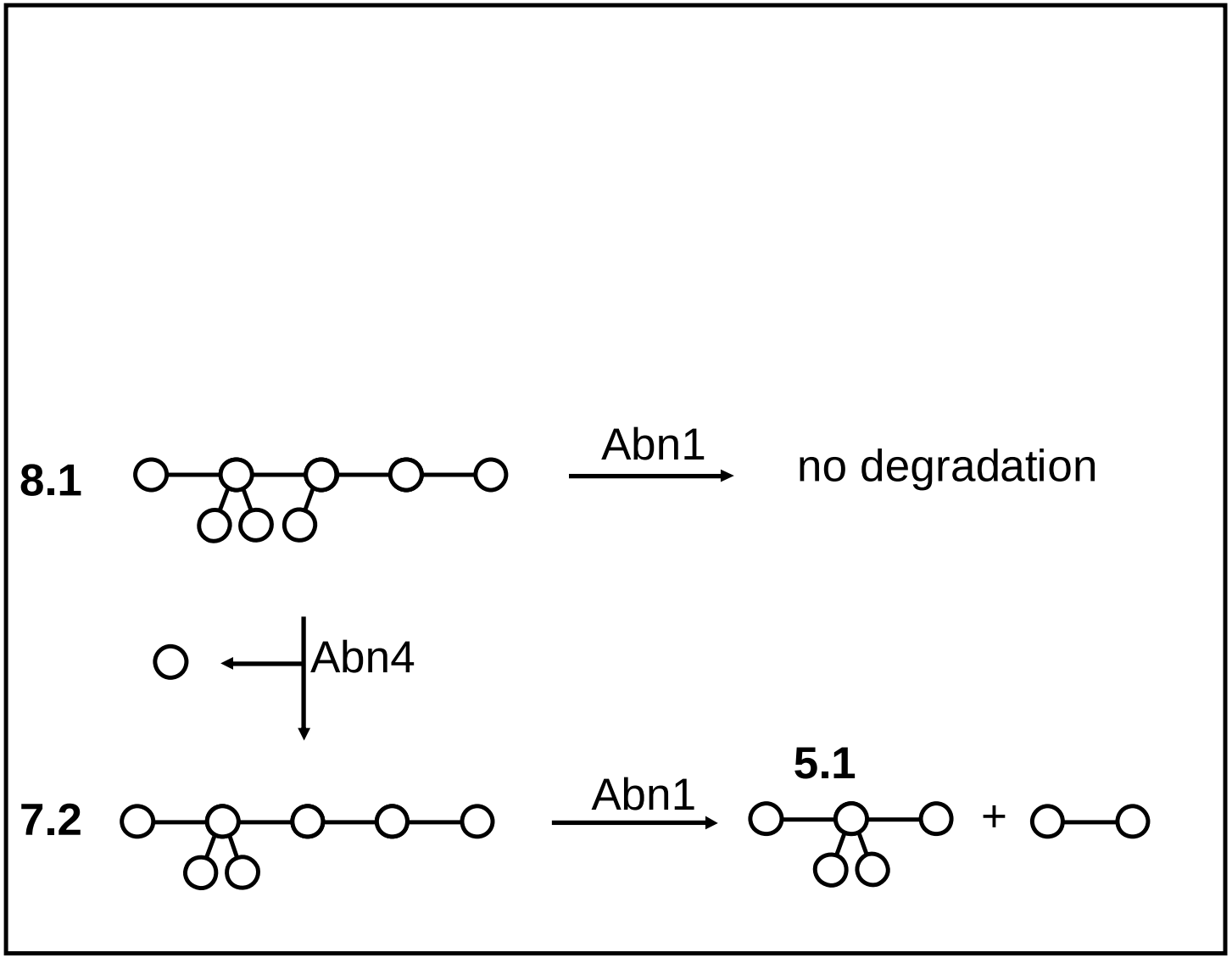

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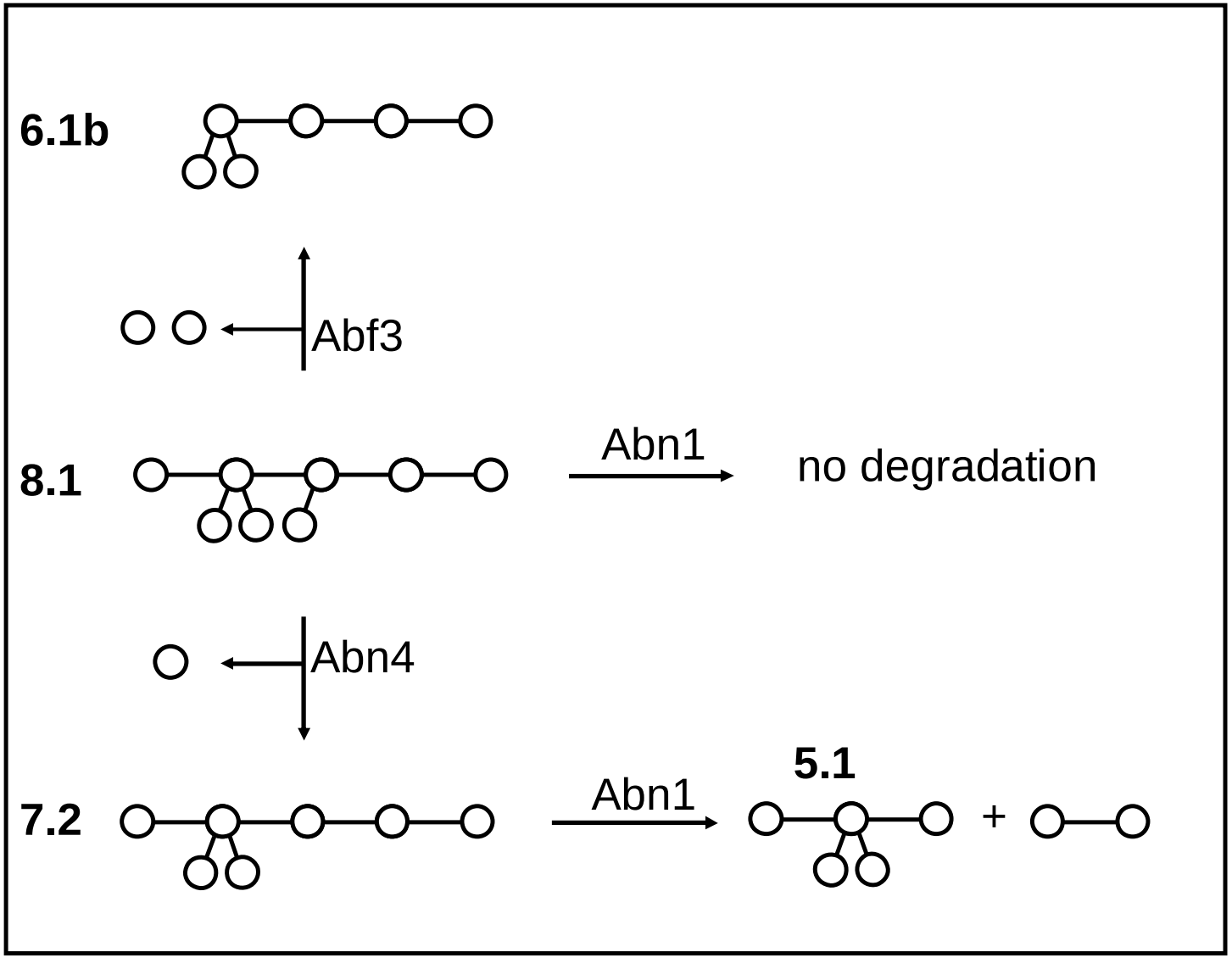

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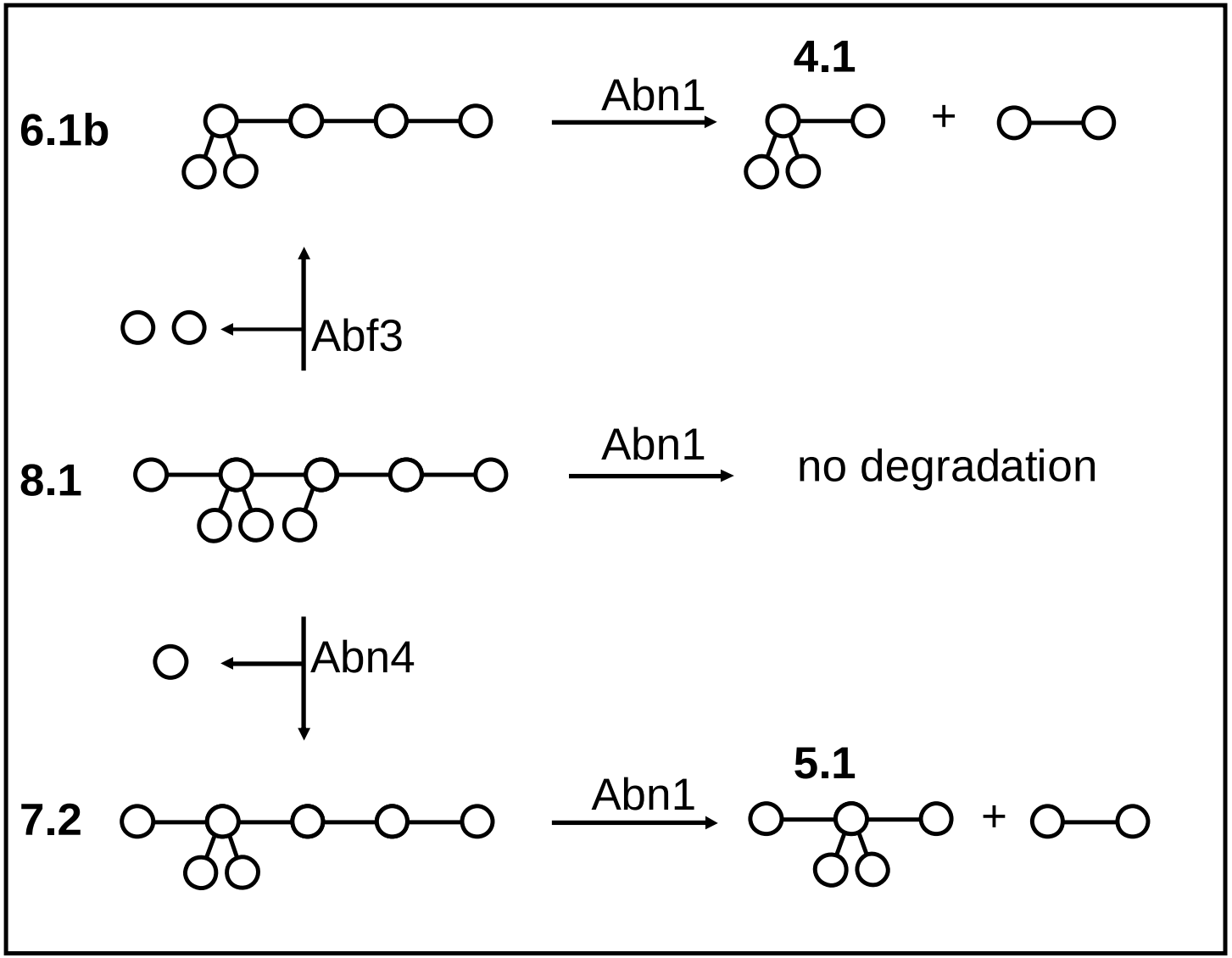

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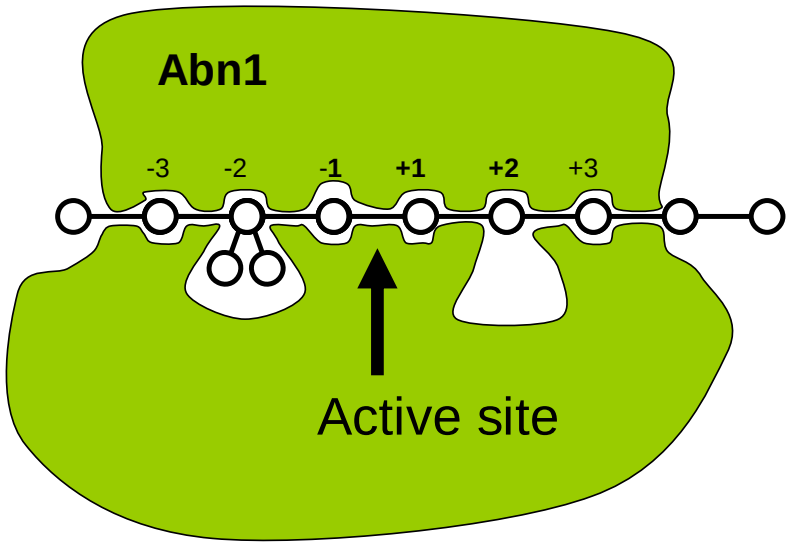
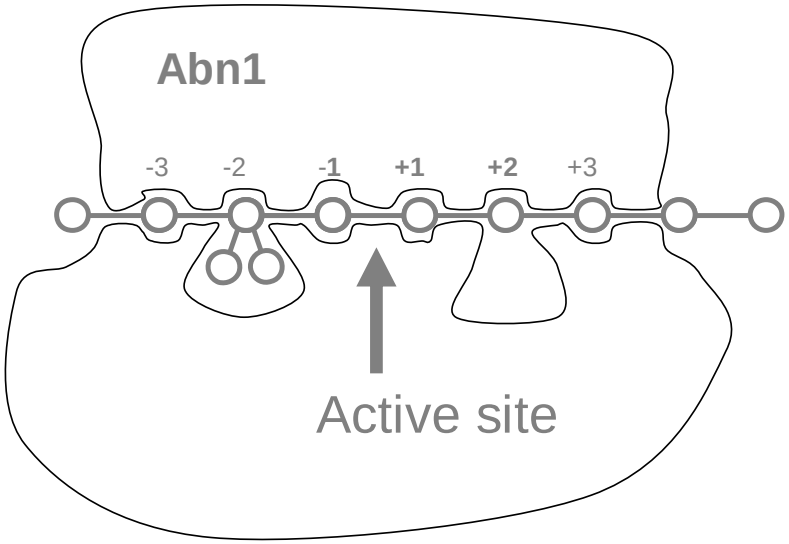
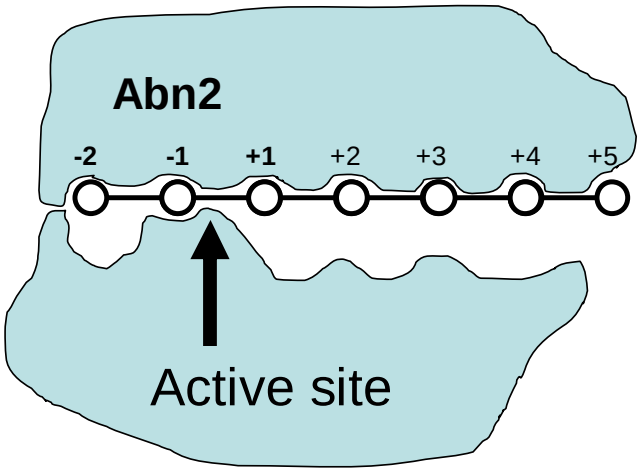
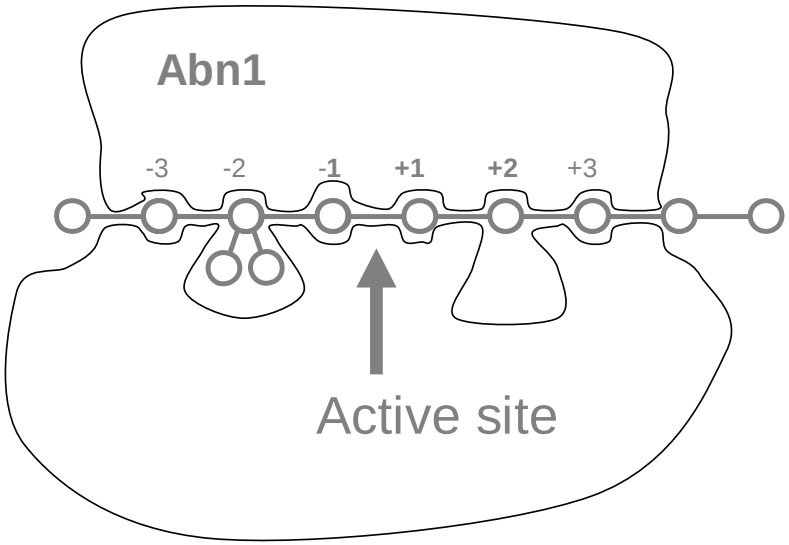


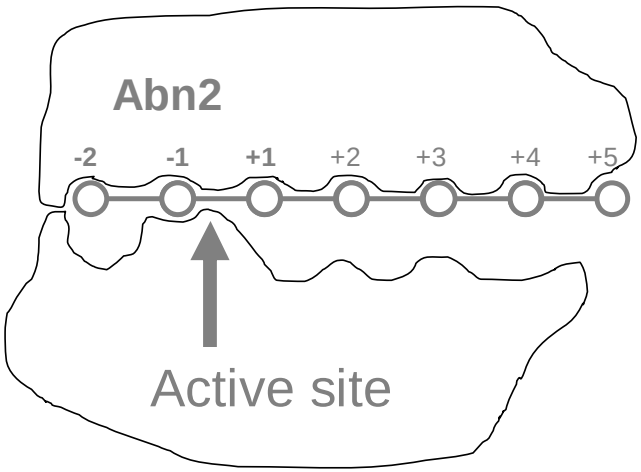
Conclusions

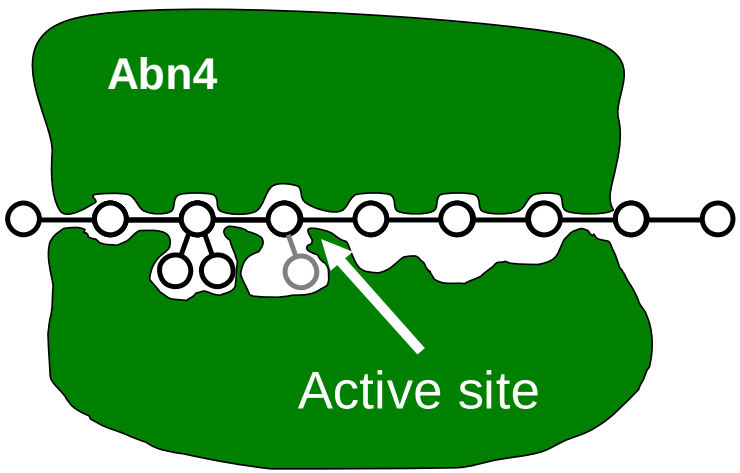




Conclusions







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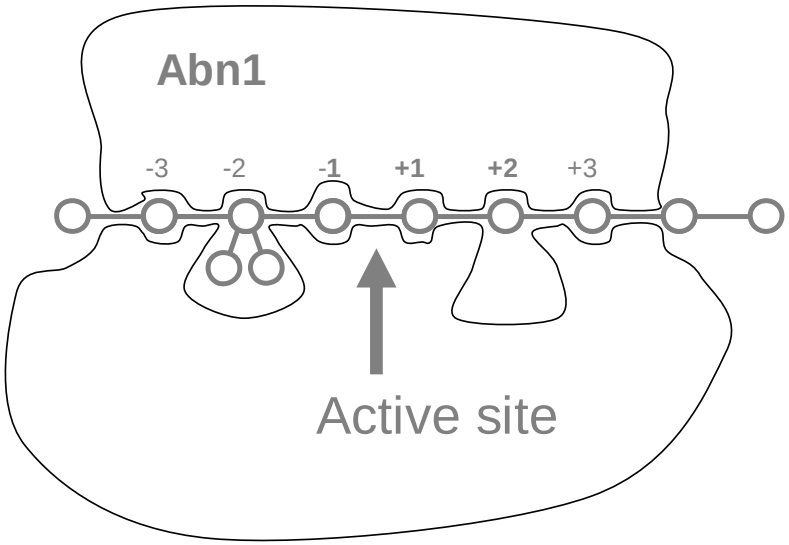
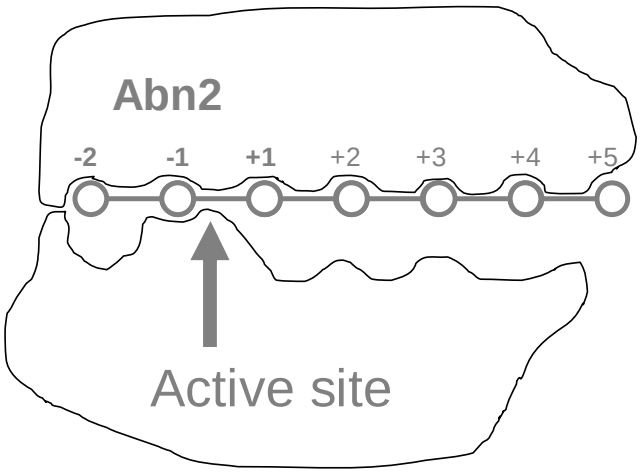
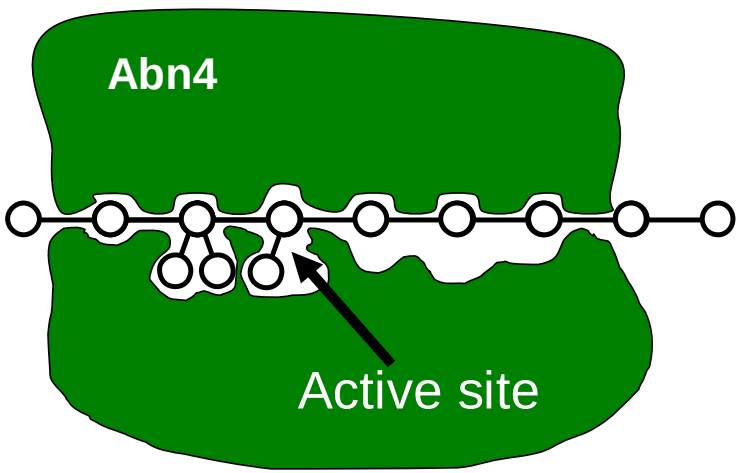
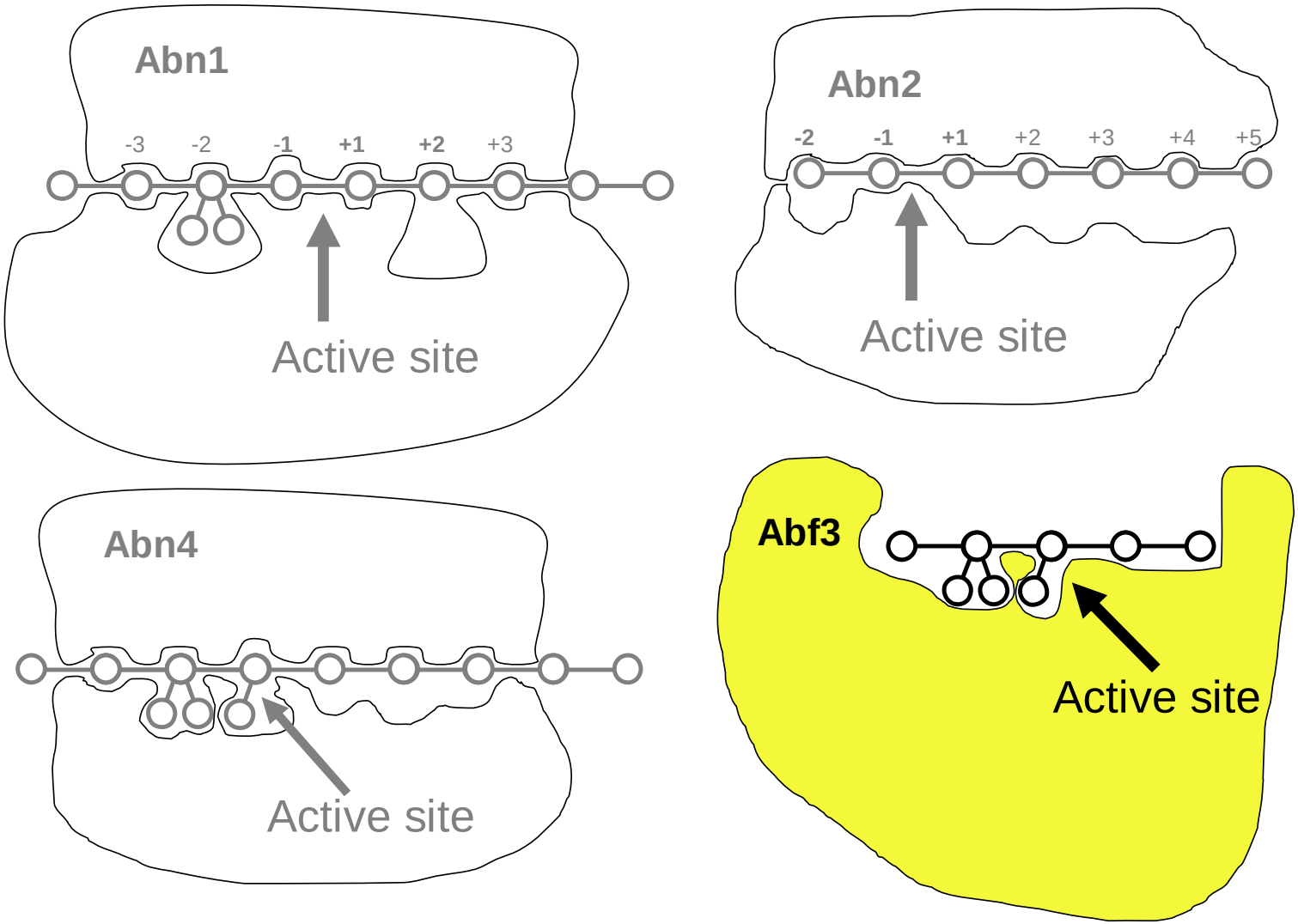






Conclusions



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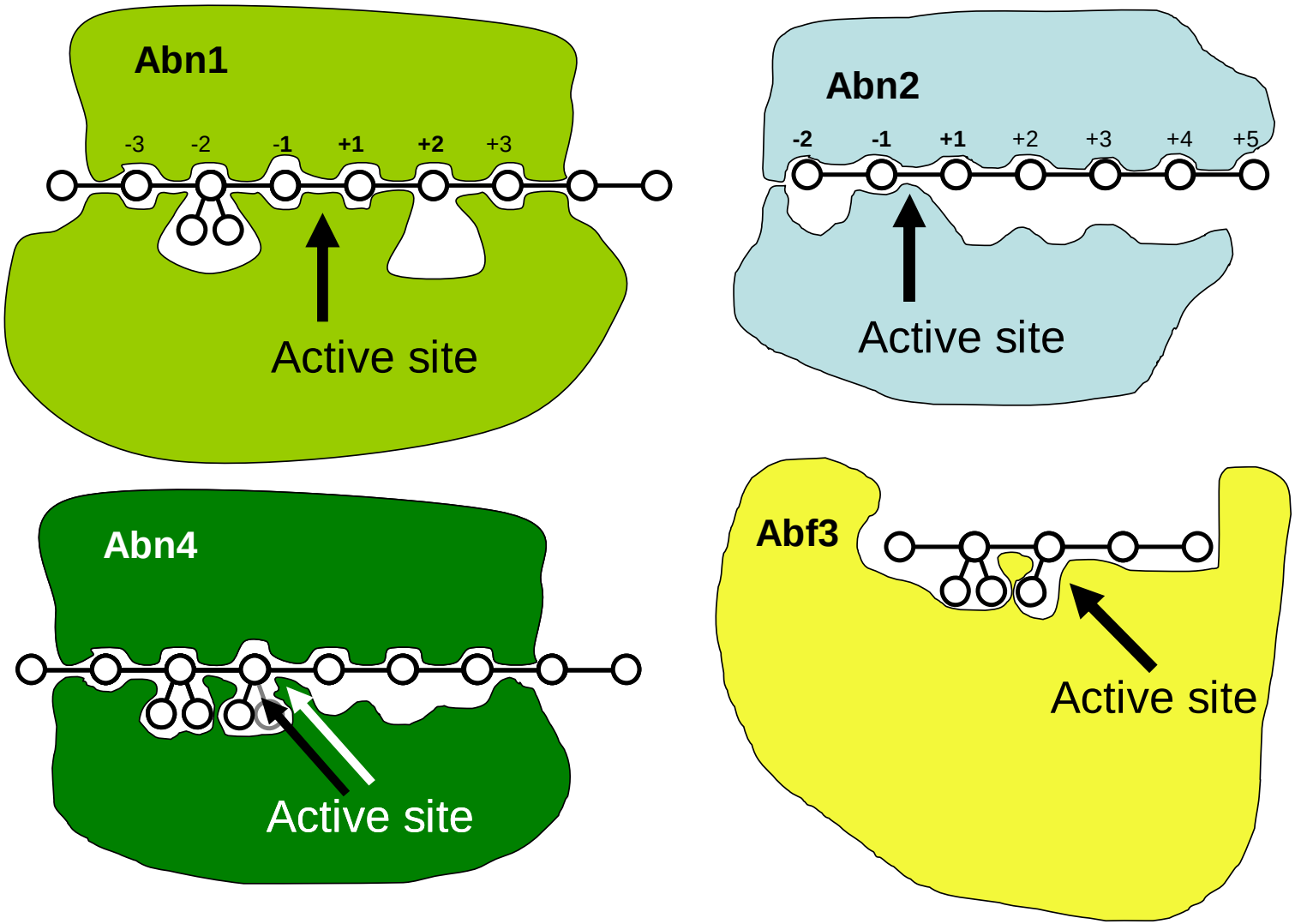

Complete enzymatic degradation of sugar beet pulp

- Identification, purification and characterisation of Ferulic acid esterases to enhance arabinan degradation from sugar beet pulp

  Kühnel *et al.* 2011, accepted for publication in *Enzyme Microbial Technology*

- Design of an enzyme mixture optimized in monomer release from pretreated sugar beet pulp

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Questions?

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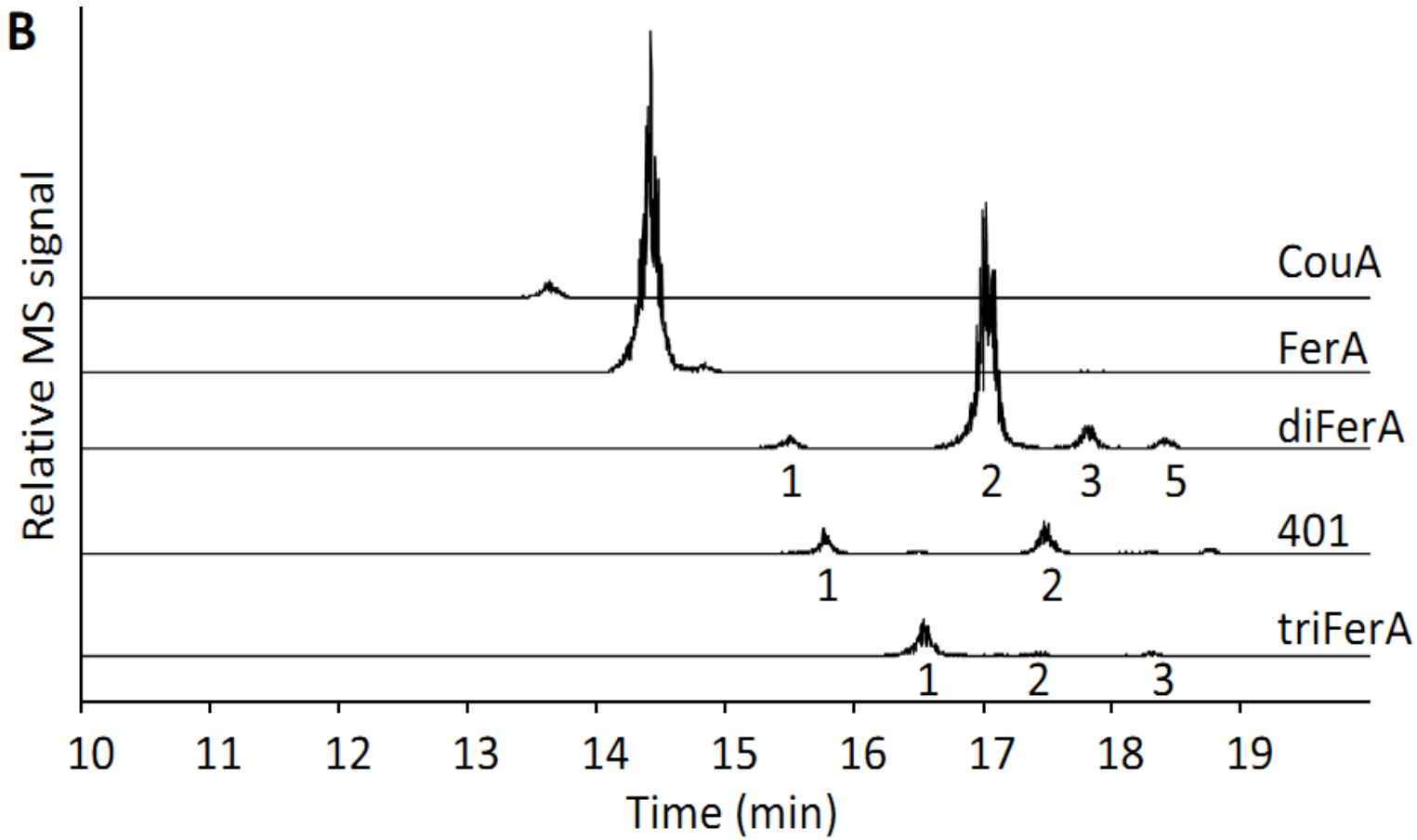
C1 ferulic acid esterases release a number of complex ferulic acid oligomers from corn fibre oligomers



A cellulase preparation can release up to 94 % of the Glucose present in pretreated sugar-beet pulp