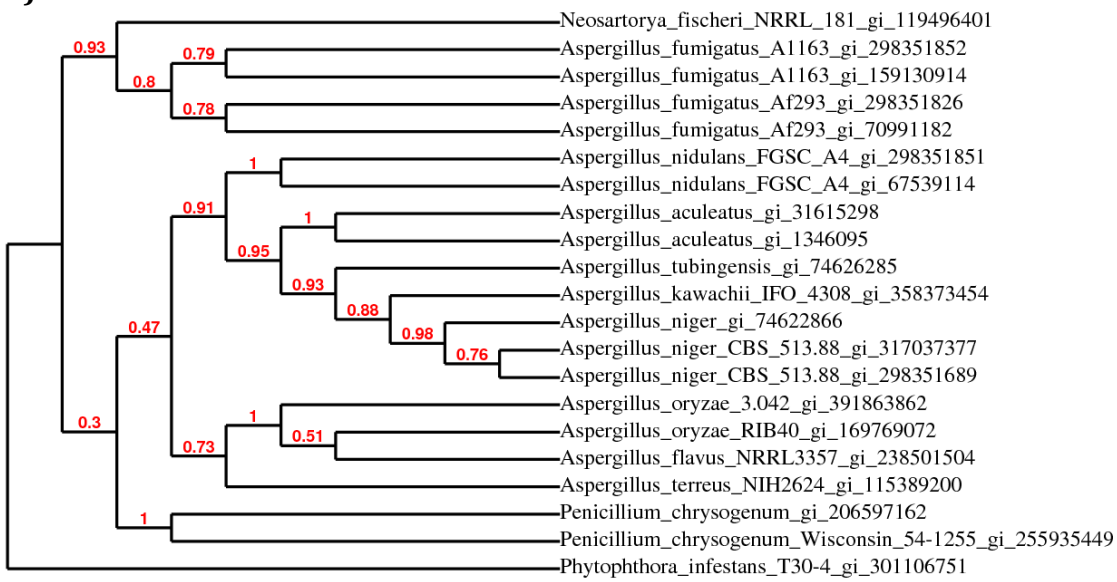
Supplementary Material

Table S1. Sequence alignment. A) Phylogenetic tree and B) sequence alignment of the known PDB structures of *endo*-β-1,4-galactanases.

A)



B)

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EnGAL    - MILSSLLPLSLVTLTSAALTYRGADISSLLIEEDSGVAYKNLNGETQAFELILANNGVNSI 61
AAGAL    -                  ALTYRGADISSLLLLEDEGYSYKNLNGQTQALETILADAGINSI
MTGAL    -                  ALTYRGVDWSSVVVEERAGVSYKNTNGNAQPLENILAANGVNTV
HIGAL    -                  ALQYKGVDWSSVMVEERAGVRYKNVNGQEKPLEYILAENGVNMV
BLGAL    -_SGTAKSGLYVEKVSGLRKDFIKGVDVSSIIALEESGVAFYNESGKKQDIFKTLKEAGVNYV _AHRD

EnGAL    - RQRIWVNPSDGSYNLEYNLELAKRVQDAGMSVYLDLHLSDTWADPGDQATPSGWSTTDIDT 122
AAGAL    - RQRVWVNPSDGSYDLDYNLELAKRVKAAGMSLYLDLHLSDTWADPSDQTTPSGWSTTDLGT
MTGAL    - RQRVWVNPADGNYNLDYNIAIAKRAKAAGLGVYIDFHYSDTWADPAHQTMPAGWPS-DIDN
HIGAL    - RQRVWVNPWDGNYNLDYNIQLARRAKAAGLGLYINFHYSDTWADPAHQTTPAGWPS-DINN
BLGAL    - RVRIWNDPYDGNNDLEKAIQIGKRATANGMKLLADFHYSDFWADPAKQKAPKAWANLNFED _ANGNGYGG

EnGAL    - LAWQVYNYTLDVCNTFAENNVAVEIVSIGNEIRNGLLHPLGSTDHYDNIARLLHSGAWGVK 183
AAGAL    - LKWQLYNYTLEVCNTFAENDIDIEIISIGNEIRAGLLWPLGETSSYSNIGALLHSGAWGVK
MTGAL    - LSWKLYNYTLDAANKLQNAGIQPTIVSIGNEIRAGLLWPTGRTENWANIARLLHSAAWGIK
HIGAL    - LAWKLYNYTLDSMNRFADAGIQVDIVSIGNEITQGLLWPLGKTNNWYNIARLLHSAAWGVK
BLGAL    - KKTALYQYTKQSLKAMKAAGIDIGMVQVGNETNGGLA---GETD-WAKMSQLFNAGSQAVR

EnGAL    - DSSLSTTPKILFHLDNGWDWDAQKYFYDTVLATGTLLSTDFDLIGVSYYPFYNADATLSSL 244
AAGAL    - DSNLATTPKIMIHLDDGWSWDQQNYFYETVLATGELLSTDFDYFGVSYYPFYSASATLASL
MTGAL    - DSSLSPKPKIMIHLDNGWDWGTQNWWYTNVLKQGTLELSDFDMMGVSFYPFYSSSATLSAL
HIGAL    - DSRLNPKPKIMVHLDNGWNWDTQNWWYTNVLSQGPFEMSDFDMMGVSFYPFYSASATLDSL
BLGAL    - ET--DSNILVALHFTN----PETSGRYAWIAETLHRHHVDYDVFASSYYPFWH--GTLKNL

EnGAL    - KTSLTNLKSNYGKNVLVVETDWPVQCSSPEYAFPSDLSSIPFSADGQETFLGRLADTLE-D 305
AAGAL    - KTSLANLQSTYDKPVVVVETNWPVSCPNPAYAFPSDLSSIPFSVAGQQEFLEKLAAVVEAT
MTGAL    - KSSLDNMAKTWNKEIAVVETNWPISCPNPRYSFPSDVKNIPFSPEGQTTFITNVANIVSSV
HIGAL    - RRSLNNMVSRWGKEVAVVETNWPTSCPYPRYQFPADVRNVPFSAAGQTQYIQSVANVVSSV
BLGAL    - TSVLTSVADTYGKKVMVAETSYTYTAEDGDNTAPKNGQTLPVTVQGQANAVRDVIQAVSDV _GHG _NN

EnGAL    - VGGVGIYYWEPGWVDNAGLGSSCEDNLMVDWRDRTVRESISVFGDL                351
AAGAL    - TDGLGVYYWEPAWIGNAGLGSSCADNLMVDYTTDEVYESIETLGEL
MTGAL    - SRGVGLFYWEPAWIHNANLGSSCADNTMFSQSGQALS-SLSVFQRI
HIGAL    - SKGVGLFYWEPAWIHNANLGSSCADNTMFTPSGQALS-SLSVFHRI
BLGAL    - G_GIGVFYWEPAWIPVGPAHRLEKNKALWETYGSGWATSYAAEYDP_               _EA

           _EDAGKWFGGSAVDNQALFDFKGRPLPSLHVFQYVDTGTPFKN
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Table S2. Geometries of the zinc binding sites.

Zinc site	Ligands	Geometry	Distance / Å
	Three protein ligands		
Zn402 (Figure 2a)	Asp149(*i*), Asp317, Asp320, OAc5	tetrahedral	Zn-O 1.69-2.09
Zn401	Glu275, Asp91(*i*), Asp332, Wat195	tetrahedral	Zn-O 1.94-2.06
	Two protein ligands		
Zn403	Glu15, Asp302, Wat198, Wat207	tetrahedral	Zn-O 1.96-2.06
Zn404 (Figure 2c)	His150, Asp152(bd), Wat224	tetrahedral	Zn-O 2.09-2.27, Zn-N 2.41
Zn405 (Figure 2b)	Lys166, Glu60(*ii*), Wat226, Wat233	tetrahedral	Zn-O 2.12-2.23, Zn-N 1.75
Zn406	Asp283, Asp287, OAcU2, OAcU3	tetrahedral	Zn-O 1.61-2.20
Zn408	His143, Glu325(*iii*), Wat216, Wat189	tetrahedral	Zn-O 1.77-2.14, Zn-N 2.08
Zn407	Glu136, Glu246, OAcN2, glycerol(td)	octahedral	Zn-O 1.99-2.06
Zn410	Asp17, Asp167(*iv*), OAcU1, OAcN3	tetrahedral	Zn-O 1.67-2.34
Zn409	Glu64(*ii*), His159, imidazoleN9, Wat211	tetrahedral	Zn-O 2.00-2.08, Zn-N 2.01-2.08
Zn411	Asp88, Asp91, Wat173, Wat174, Wat180	trigonal bipyramid	Zn-O 1.97-2.11
Zn412	Asp185, Glu28(*iii*), OAcN6, Wat214	tetrahedral	Zn-O 1.91-2.16
	One protein ligand		
Zn415	Asp102, Wat143, undefined water	143°	Zn-O 2.03-2.09
Zn413	Glu286, Wat165, Wat183, Wat184	tetrahedral	Zn-O 2.08-2.27
Zn414 (Figure 2c)	His150, Wat234, Wat235, Wat236		Zn-O 2.28-2.53, Zn-N 2.34

i –x, y+1/2, -z-1/2
ii x-1/2, -y+1/2, -z
iii –x, y-1/2, -z-1/2
iv x+1/2, -y+1/2, -z
bd bidentate
td tridentate

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Figure S3. Binding of a zinc ion in the active site.

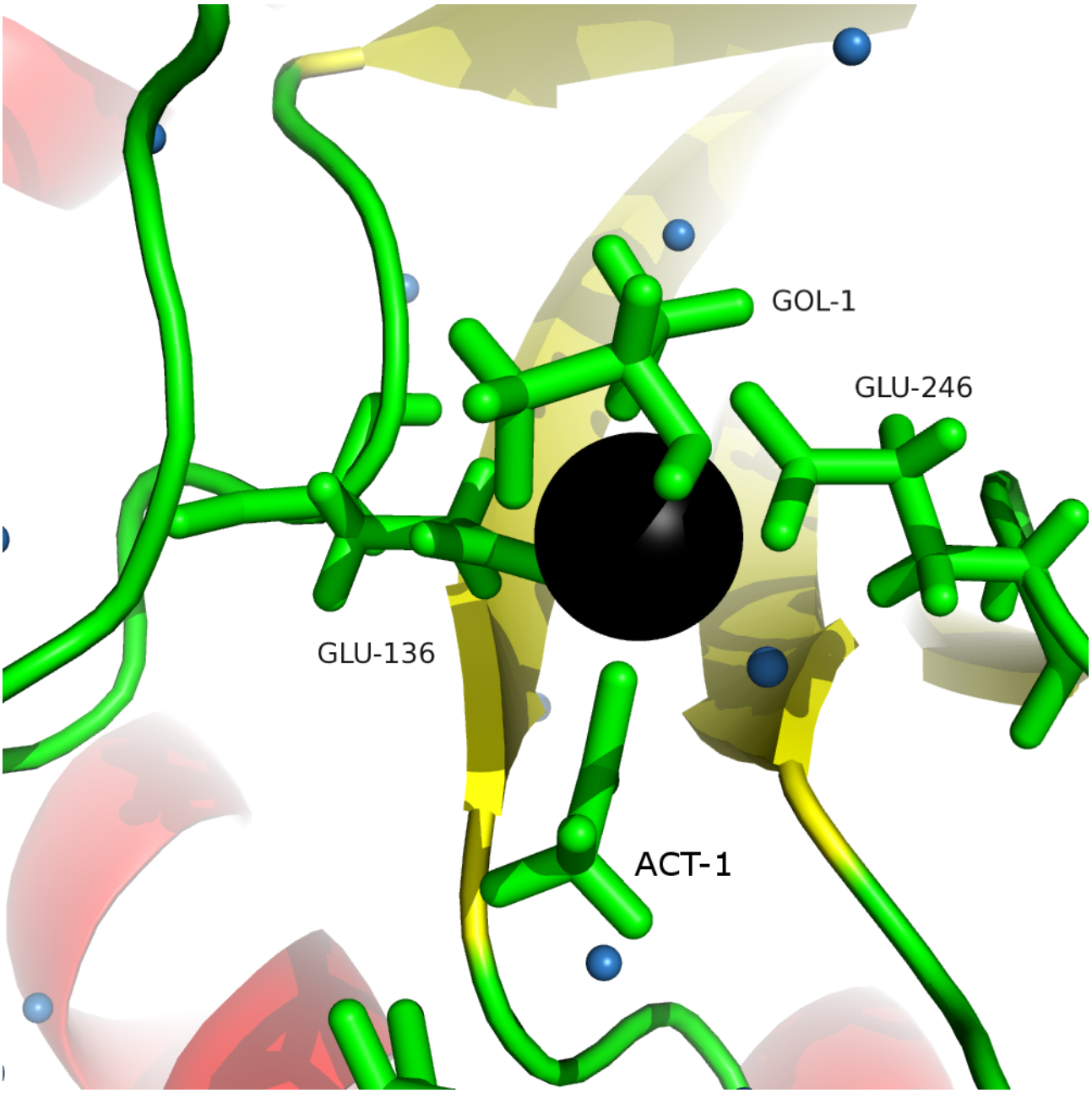

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Figure S4. Crystal packing mediated by zinc ions. Tetrahedral coordination of EnGAL monomers within in the crystal packing of the orthorhombic cell.