

## SUPPLEMENTARY INFORMATION

Variations of the NodB architecture are attuned to functional specificities into and beyond the CE4 family

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**Table S1.** NodB-domain containing CE4 proteins extracted from the Protein Data Bank before July 2023. Our analysis was performed at the non-redundant NodB-domains of the underlined structures. PG, DA and MD stand for Peptidoglycan, DeAcetylase and Molecular Dynamic simulations, respectively.

Subgroups of CE4 family	Protein name/family	PDB code	Organism	Activity data
Chitin/Oligochitin DA	CICDA	<u>2IW0</u>	<i>Colletotrichum lindemuthianum</i>	Active on (GlcNAc) <sub>3-6</sub> and partially deacetylated chitooligosaccharides
	AnCDA	<u>2Y8U</u>	<i>Aspergillus nidulans</i>	Active on (GlcNAc) <sub>2-6</sub> , chitosan, crystalline chitin, acetylxylin. Inactive on GlcNAc, peptidoglycan
	VpCOD	<u>3WX7</u>	<i>Vibrio parahaemolyticus</i>	Active on (GlcNAc) <sub>2-4</sub>
	VcCOD	4NY2, 4NYU, 4NYY, 4NZ1, 4NZ3, 4NZ4, 4NZ5, 4OUI	<i>Vibrio cholerae</i>	Active on (GlcNAc) <sub>2</sub>
	ArCE4	<u>5LFZ</u> , 5LGC	<i>Arthrobacter species</i>	Active on (GlcNAc) <sub>3-6</sub> , chitosan, $\alpha$ - and $\beta$ -chitin, acetylxylin. Low activity on (GlcNAc) <sub>2</sub>
	BmCDA8	<u>5Z34</u>	<i>Bombyx mori</i>	Active on (GlcNAc) <sub>3-6</sub>
	BmCDA1	<u>5ZNT</u> , 5ZNS	<i>Bombyx mori</i>	Deacetylates ethylene glycol chitin in the presence of accessory proteins
	AngCDA	<u>7BLY</u>	<i>Aspergillus niger</i>	Active on GlcNAc <sub>2-6</sub> , weak activity on $\alpha$ -chitin and $\beta$ -chitin, slight increase on colloidal chitin
	Pst_13661	<u>8HF9</u> , 8HE1, 8HE2, 8HE4	<i>Puccinia striiformis</i>	Gene knock-out makes it inactive against chitin.

	VdPDA1	<u>8HFA</u>	<i>Puccinia striiformis</i>	Gene knock-out makes it inactive against chitin
<b>PG GlcNAc DA</b>	SpPgdaA	<u>2C1G</u> , 2C1I	<i>Streptococcus pneumoniae</i>	Active on peptidoglycan
	Bc1960	<u>4L1G</u> , 5O6Y	<i>Bacillus Cereus</i>	Active on peptidoglycan (from gram <sup>+/−</sup> bacteria), N-acetyl-D-glucosaminyl-( $\beta$ -1,4)-N-acetylmuramyl-L-alanyl-D-isoglutamine, (GlcNAc) <sub>4-6</sub> , glycolchitin
	Bd3279	<u>5JP6</u>	<i>Bdellovibrio bacteriovorus</i>	Active on peptidoglycan
	Bc1974	<u>5N1J</u> , 5N1P, 5NC6, 5NC, 5NCD, 5NEK, 5NEL	<i>Bacillus Cereus</i>	Active on (GlcNAc) <sub>5</sub>
<b>PG MurNAc DA</b>	BsPdaA	<u>1W17</u> , 1W1A, 1W1B, 1NY1	<i>Bacillus subtilis</i>	Active on peptidoglycan. Protein/NAG complex (crystallography).
	BaCE4	<u>2J13</u>	<i>Bacillus Anthracis</i>	Putative activity. No biochemical characterization is provided; however, the protein is considered to be the <i>B. anthracis</i> ortholog of BsPdaA.
<b>PG MurNAc &amp; oligochitin DA</b>	BsPdaC	<u>6H8L</u> , 6H8N	<i>Bacillus subtilis</i>	Active on (GlcNAc) <sub>4-5</sub> & chitooligosaccharide kinetics
<b>Acetylxyloxy esterases</b>	SICE4 (SIAXe)	<u>2CC0</u>	<i>Streptomyces lividans</i>	Active on 2-O-acetyl-4-nitrophenyl -D-xylopyranoside
	CtICE4 (CtAXe)	<u>2C71</u> , 2C79	<i>Clostridium thermocellum</i>	Active on 2-O-acetyl-4-nitrophenyl -D-xylopyranoside
	XylICE4	<u>7AX7</u>	<i>Trinervitermes trinervoides</i>	Active on p-NPA (4-nitrophenyl acetate)
<b>Poly-<math>\beta</math>-1,6-GlcNAc DA</b>	AdIcaB	<u>4WCJ</u>	<i>Ammonifex degensii</i>	Active on ( $\beta$ -1,6-GlcNAc) <sub>4</sub> and ( $\beta$ -1,6-GlcNAc) <sub>5</sub>
	EcPgaB	3VUS, <u>4F9J</u> , 4F9D, 4P7L, 4P7N, 4P7O, 4P7Q, 4P7R	<i>Escherichia coli</i>	Active on $\beta$ -1,6-GlcNAc oligomers
	AaPgaB	<u>4U10</u>	<i>Aggregatibacter actinomycetemcomitans</i>	Active on 7-acetoxycoumarin-3-carboxylic acid
	BpsB	<u>5BU6</u>	<i>Bordetella bronchiseptica</i> periplasmic	Active on $\beta$ -1,6-(GlcNAc) <sub>1-5</sub>
<b>PG GAC linkage DA</b>	SpPplD	<u>6DQ3</u>	<i>Streptococcus pyogenes</i>	de-N-acetylase of the cell wall linkage of streptococcal rhamnopolysaccharides
	SmCE4	<u>2W3Z</u>	<i>Streptococcus mutans</i>	Active on (GlcNAc) <sub>6</sub> . No activity was observed toward shorter chitooligosaccharides or a synthetic peptidoglycan tetrasaccharide. Authors suggest activity on as-yet unidentified polysaccharide.
	Bc0361	<u>4HD5</u>	<i>Bacillus Cereus</i>	-
	Ba0330	<u>4V33</u>	<i>Bacillus Anthracis</i>	Inactive on glycol chitin, GlcNAc <sub>2-6</sub> , p-nitrophenyl acetate, N-acetyl-D-

Substrate identification awaits experimental confirmation				glucosaminyl-( $\beta$ -1,4)-N-acetylmuramyl-L-alanyl-D-isoglutamine
	Ba0331	<u>6GO1</u>	<i>Bacillus Anthracis</i>	-
	PAO1	<u>1Z7A</u>	<i>Pseudomonas aeruginosa</i>	-
	HpPgda	<u>3QBU</u> , 4LY4	<i>Helicobacter pylori</i>	Inactive on GlcNAc, GlcNAc <sub>3</sub> , N-acetyl putrescine, N-acetyl spermidine, N-acetyl cadaverine, N-acetyl dipeptides, allantoin
	Bb0128	<u>3HFT</u>	<i>Bordetella bronchiseptica</i>	-
	MsCE4	<u>3RXZ</u>	<i>Mycobacterium Smegmatis</i>	-
	BpCE4	<u>3S6O</u>	<i>Burkholderia pseudomallei</i>	-
	Bacova_03992	<u>4DWE</u>	<i>Bacteroides ovatus</i>	-
	EcCDA	<u>2VYO</u> [Error! Reference source not found.]	<i>Encephalitozoon cuniculi</i>	Inactive on chitooligosaccharides and crystalline $\beta$ -chitin. EcCDA exhibited no de-N-acetylase activity on neither of the substrates tested
	ErPgda	<u>5JMU</u>	<i>Eubacterium rectale</i>	-
	CsCE4	<u>7FBW</u>	<i>Caldanaerobacter subterraneus</i>	-
	ATU2773	<u>2QV5</u>	<i>Agrobacterium tumefaciens</i>	-
Pseudo deacetylases	Ba0150	<u>4M1B</u>	<i>Bacillus Anthracis</i>	No activity
	Ba3943	<u>6HM9</u> , 6HPA 7BKF	<i>Bacillus Anthracis</i>	-



MHNGIRMTTLVKRAMLVCAGVLFTYEVAFGSVHTALASTEDEAKSVQLVSEIQTSLAPKEAPKHYNGQVRKVAYLTFDDGPGKYTAE  
LLDMLKKENAKATFFLIGSNVKAFPDLVKREDAEGHYVGMHSMTHNYKKLYTEGHYVDEMKEQGLIAGVLGKSPVLTRPSYSGMP  
GLNEALRNKVVENGLKVDWDWTDISLDWKYNKMQVDAASAKIVENVLHGATNPTEVILMHDHQPVSVAVPGIIGKGLKEKGYLEAYN  
ENEHFPLNFWHDNRM

>BC5204

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>BsPdaC

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>CdPdaV

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>SpPgdA

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LMRPPYGAITDDIRNSLDLSFMWVDVSLDWKSKNEASILTEIQHQVANGSIVLMHDIHSPTVNALPRVIEYLNKNGQYTFVTIPEMLNTR  
LKAHELYYSRDE

>SsPgdA

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AQGMKETDVEAIVKKFETLDLNAISFSYGDSQLTLQLPDGYGINQLVLPISDLYPVVKSDYLVADAKVGYDEYMAAQVVDKHLRQV  
ALTFDDGPNPNTTPVVDLLKKYNAKATFFVVGKAVVGNEAILRRMVAEGHVIANHTWNHPNLVTISGEQVQREIQDTQAAITEATGI  
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>LiPgdA

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LIALTFDDGPNPNTTPQLLKIFSDAQVPATFFALGKQAQACPQIIEEADRGNEVASHTWDHKDLVTLSPDQKQIEESANQLINKITGN  
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>EfPgdA

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>Bd0468

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>Bd3279

MYMKKL VFGGMLIVSAASLVGCGSQIGSSVRQAVSDNQSAQTLVWENSEANPEALFANWRHEFMVDSSKRESMKTELCKELQALPQ  
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DDGPHGLYTDAILRALKEVNAKAMFFATGKSVRTNPEALKRVAADGHVIGSHSITHACLGTSVACYKQMGNRNLTFDEAAA EVRGGQ  
AVFDVLGWVDPVFRFPYGETSKDLKAFKTKSTGEFAWNIESDDWRTQSNQELLARVLANVESQGRGIVLFHDIQRRTAEIMPQFLREL  
YNRGYSVVLLTAADPSAKYNSKLVKRKQQLP

## MurNAc PG deacetylases:

>BsPda-1W17

MKWMCSICCAAVLLAGGAAQAEAVPNEPINWGFKRSVNHQPPDAGKQLNSLIEKYDAFYLGNTKEKTIYLTDFDNGYENGYPKVLVDV  
LKKHRVTGTTFFVTGHFVKDQPPQLIKRMSDEGHIIGNHSFHHPDLTTKTADQIQDELDSVNEEVYKITGKQDNLYLRPPRGVVFSEYVLKET  
KRLGYQTVFWSVAFVDWKINNQGKGGKYAYDHMIKQAHPGAIIYLLHTVSRDNEALDDAITDLKKQGYTFKSIDDLMEFEKEMRLPSL

>BsPdaC

MLAKRIKWFWHLIAVVCVVVLIGFFHNHSLKKETVMNKKVRTDSQYGNVEIATLVNDGKTFNYAVNYPVFKNEKMDSALKRFAEKEVR  
QFQKETKDVDQEHTTKRNLNVDYKIVHYAKQTVAIVFNEYKYIGGAHGQTVKKTFNYDFSKQAFLSIDDIFKEDADYLHKLSLIAYHE  
LKKNKDIAADDALLKEGTAPKKENFSRFAIKEDYIELYFDITYQVAAGYLGEQSAIKKSLKDILKEQYIDKAKNKNKIKEQKPKHEVIS  
LPKEETVDPNQKVIALTFDDGPNPATTNQILDLSKKYKGHATFFVLGSRVQYYPETLIRMLKEGNEVGNHNSWSHPLLTRL SVKEALKQI  
NDTQDIIKISGYRPTLVRPPYGGINDELRSQMKMDVALWDVPEDWKDRNKKITVDRVMNQAGDGRITLHDIYRTSADAADIEIHKKT  
DQGYQLVTVSQLEEVKKQREAK

>CIPdaA1

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LNNKK

>CIPdaA2

MFRKVLHYLTLLSISIFFIVGCSNSQNNQNNENQNKETQLQEDKEKIDSGKDTSNVIVSDGTDKPSKATTNNDNNKLDVSSLDNTTLDWF  
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HIVGNHSKTHPSMPTKSNLKNFNDELYDVEKLYKDVGTGKDMVKFFRPPMGKYSEKSLAMTKNLGYKTVFWSFAYRDWDTDKQPSH  
EATQKIMDNLHDGSILLHLHAVSKTSTEILNDFISNARKLGYEFELLEY

>BaCE4-2J13

MAYTNTPHNWGIPRPKNETVPDAGKLYTDLLQKNGGFYLGDTKKKDIYLTDFDNGYENGYTGKILDVLKEKKVPATFFVTGHIYKTQDL  
LLRMKDEGHIIGNHSWSHPDFTAVNDEKREELTSVTTEEIKKVTGQKEVKYVRPPRGVFSERTLALTKEMGYYNVFWSLAFLDWKVDE  
QRGWQYAHNNVMTMIHPGSILLHLHAISKDNEALAKIIDDLREKGYHFKSLDDLKSNQP

## Chitin/oligochitin deacetylases:

>CICDA-2IW0

MHFSTLFGAAATAALAGSTNASPLARRQVPVGTPILOCTQPLVALTYDDGPFTFTPQLLDILKQNDVRATFFVNGNNWANIEAGSNPD  
TIRRMRADGHLVGSHTYAHPDNLTLSSADRISQMRQLEEATRRIDGFAPKYMRAPYLSCDAGCQGDGLGGLGYHIIDTNLDTKDYENNK  
PETTHLSAEKFNNELSADV GANSYIVLSHDVHEQTVVSLTQKLIDTLKSKGYRAVTVGECLGDAPENWYKA

>5LFZ-ArCE4

AGQPEPVATPPAVDCATTCKVALTFDDGPGEYTNRLLEDELSEQHTPATFFVLGKNVKKYPKTLKRMVDEGHQIGSHTFDHKDITKLTA  
EGIEHEVQWTDEAIEQAAGVKPQILRPPYGAHGAVYDRLIPYPLVLWDVDTLDWKHHPQKTVRIALEEAKPGSIILMHDIHESSVKA  
PQLVSKLHDAGYTLVTVDQLFAGTDFKPAKAYDHRFKTNP

>5Z34

TELPLATPCDEEACKLPDCRCSSTNIPGGLRARDTPQFVTVTFFDDGINVINIETYREVLYGRSNSNRCPAGATFYVSHEYTNYQLVNELY  
NRGFEIALHSISHRTPQAFWADATYQNLVQEIGDQKRQMAHFASIPASAIKGVRIPLQMSGNTSFQVMADFDLLDYCTWPTALTNP  
LWPTYTLHHESIQCIIPPCTASIPGPWVLP MISWRDLNFPSCSMVDGCFPTPDRTDDEGWFKFILTNFERHYLGNRAPFGFFVHEWFISS  
NPAIKRAFVRFMDIINLNDVFMVNSAEVIDWVKNPVPIDRYRQQQCKFTMPSICRPSFCGPLTGTHNQLSYYMTICNTCPRNPYPWVG  
NPLGQ

>A.nidulans CDA

MLFPNVPGVASLLSLFAATLILNTQALAINLTDRQPRVSYGLYIHHCYVPGVVALTFFDDGPYIYTEELLDILAQYGAKATFFVNGH  
NLAGNEWLIQRVVNEGHLASHTWGHDTLTVLSYDQIVDMQTRLESFAFVASVGVVPTYMRPPYLAANDYVLGVMAELGYHVIGASV  
DTKDYENDHPDLIGRSVAKFNQELDQGGTIVLSHDIHEQTVRTLTHIMLEEVYERGLQPTTVGGCLGDDAWYR

>R.circinans CDA1

MVWKTAFSAIAIASVNAVTTNFTSKTDPTNITIPAIEQTTSHPVKECTSYQSPYTINQAEWPTSWTTATSNMGMTSAEFQALYNSIDWT  
KAPNFPVRKLSATTGGLDMTGYDTATDPACWWSSTCTKPKTEGINADLYACPEPDVWGLTFDDGPNCSHNAFYDYLEQNKLKASMF  
YIGSNVLNWPYGALRGVKGHHIAGHTWSHQLMTTLTNQEILAELEYTQKAIKYVTGITPKYWRPAQGDIDDRVRWIATQLNLTAIW  
NLDTTDDWAAGTTPGITAETVNQNYEDFIKMGSNGTANTGNVLSHEINNMTMSFFMNHYEIEIKAYTHVLVDVATCMNITNPYQETTI  
TFPTFDQAINGASVSTSNGSSTSASAKSAGTSVQLNASLLVAALMGLMVL

>R.circinans CDA2

MVWKTAFSAVAIASVNAVTTNFTSTVDPANITIPAIQTTSLDPVKECTSYQAPFAFDQKEWPTNWDVATSNMGMTSAEFQALYKSID  
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AILWNLDTDDWAAGTTPGITAETVSQNYEDYIKMGSNGTANTGNVLSHEINNMTMGFFLDHYEIEIKAYTHVLVDVATCMNITNPY  
ETTPTFTFEQAIGGASAFASNGFFNGTSPLSVAASKSAGAPIQLNGSLLVAALMGLLVFA

>R.circinans CDA3

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EATSNGMTKTSEFQALNKSIEWSKAPKIPVRKAGSDGSLDMTKYSDDPDCWWSSTCTKPKHKDINEDIYACPEPETWGLTYDDGPN  
CSHNAFYDYLEQNKLKASMFYIGSNVNVNWPYGAQRGVKAGHHIADHTWSHQLMTTLTNDEVLAELEYTQKAIKMVTGVTPLYWRPA  
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KNVVDVATCMNITYPYQEHNVSFAPFGSAADESTATSTNATASSASASAAATSEDEPGTTVIPLAANKAQIASAGIQVNPNSLVFAAFVA  
AAYFF

>S.cerevisiae Cda2p

MRIQLNTIDLQCIIALSCLGQFVHAEANREDLKQIDFQFPVLERAATKTPFPDWLSAFTGLKEWPGLDPPIPLDFIDFSQIPDYKEYDQN  
HCDSVPRDSCSFDCHHCTEHDDVYTCSKLSQTFDDGPSASTTKLLDRLKHNSTFFNLGVNIVQHPDIYHRMQKEGHLIGSHTWSHVYLP  
NVSNEKIIAQIEWSIWAMNATGNHTPKWFRPPYGGIDNRVRAITRQFGLQAVLWDHDTFDWSSLNDSVITEQEILQNVINWNKSGSGL  
ILEHDSITEKTVDLAIKINKLIGDDQSTVSHCVGGIDYIKEFLS

**Poly-β-1,6-GlcNAc deacetylases:**

>AdIcaB-4WCJ

SSGLVPRGSHMESPRTPAGTHLQGDGLVVLGYHRVLPSSRYAISRREFAQQLDYLRQVGVRVTPQEAEDYLAGRIHLPGLVLTFFDD  
GDLSVYRHAFFVLKRRKIPFLFFVIAGQVGRKWEFGSMCSWEQIKEMVASGLCVVGLHTYDLHYWDSQAKKPVFLLPGRERLFAEDT  
ARGTACLKEHLGLKTRYFAYPYGFGTPTTDEILRTQGFSLVFTLRKAVNRPGDAPFVGRVLTPTDSWPQVAAWAQASP

>SeIcaB

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