Chitinases

Identity, structure & kinetics

Chitinase candidates (hydrolyse chitin -> chito-oligosaccharides)

GH18 enzymes need an acetylated -1 subsite; most produce (GlcNAc)2-dominant products. Where the polymer is deacetylated first, the chitinase is replaced with a chitosanase (bottom rows).

Enzyme	Class / EC	Source organism	RG	Accession (UniProt / GenBank)	PDB	Size	Domain architecture	kcat	Km	Substrate (for kinetics)
SmChiA (WT)	Endo/exo-chitinase; GH18; EC 3.2.1.14 / 3.2.1.201	Serratia marcescens	RG1	UniProt P07254; GenBank AJ630582 (BJL200 operon)	1CTN	~563 aa; 50-52 kDa	N-term FnIII-like + (b/a)8 TIM-barrel catalytic + small a/b insertion (chitin-binding)	33 +/- 1 s^-1	9 +/- 1 uM	(GlcNAc)4 (HPLC natural-substrate assay)
SmChiB	GH18; EC 3.2.1.14	Serratia marcescens	RG1	UniProt P11797	varied	~55 kDa	Catalytic TIM-barrel + C-term chitin-binding domain (opposite polarity to ChiA)	28 +/- 2 s^-1	4 +/- 2 uM	(GlcNAc)4
SmChiA F232W/F396W	GH18 engineered variant	Serratia marcescens	RG1	mutant of P07254	-	~563 aa	As SmChiA; two Phe->Trp at cleft entrance/exit	Higher than WT on crystalline chitin	Not reported	crystalline (alpha) chitin
ChiA (strain B4A)	GH18; EC 3.2.1.14	Serratia marcescens B4A	RG1	strain-specific (see ref)	-	~50 kDa	ChiA-type multidomain	134.75 min^-1 (~2.25 s^-1)	3.72 mg/mL	colloidal chitin
Acid chitinase (chitobiosidase)	GH18; EC 3.2.1.14	Rasamsonia emersonii / Rhizopus oryzae	RG1	see ref	-	46-48 kDa (glycosylated)	GH18 catalytic + substrate-insertion domain	Not reported	Not reported	Chitin / colloidal chitin
Bacillus / Streptomyces GH18 chitinase	GH18; EC 3.2.1.14	Bacillus spp. / Streptomyces spp.	RG1	search CAZy GH18 (many)	many	varies	Catalytic TIM-barrel +/- ChBD (CBM12/CBM5)	enzyme-dependent	enzyme-dependent	colloidal chitin / (GlcNAc)n
Chitosanase A / B (deacetylate-first route)	GH; chitosan endo-hydrolase	Mucor rouxii	RG1	see ref	-	76 & 58 kDa (A / B)	Endo-acting chitosanase catalytic domain	Not reported	Not reported	chitosan (DD 70-100%)
ChiC1 (endochitinase C)	GH18 endo-chitinase; EC 3.2.1.14	Serratia marcescens BJL200	RG1	UniProt Q068W1 (Horn 2006 dataset - verify); gene chiC	ChiC2 cat. domain (Payne 2012)	~52 kDa	(b/a)8 TIM-barrel catalytic (shallow cleft, no processivity aromatics, Ca2+ site) + FnIII + CBM12	~30x lower than ChiA on 4-MU-(GlcNAc)3 oligomer; but 10-15x faster than ChiA/ChiB on polymeric CM-chitin	higher than ChiA on oligomer	4-MU-triacetylchitotrioside / beta-chitin
ChiC2 (catalytic domain only)	GH18 endo-chitinase; EC 3.2.1.14	Serratia marcescens (truncated form)	RG1	catalytic module of ChiC (Payne 2012)	Cat. domain solved (Payne 2012)	~28-36 kDa	(b/a)8 TIM-barrel catalytic only (no FnIII / no CBM) -> compact, fusion-friendly	endo; similar profile to ChiC1 on soluble substrate	-	beta-chitin / soluble chitin
PpChi	GH18 chitinase; EC 3.2.1.14	Paenibacillus pabuli	RG1	GenBank OME85809.1; gene BK122_02780	-	~region GH18	GH18 catalytic + CBM	67.2 s^-1	see ref	partially acetylated chitosan (DA ~48%) - not fully acetylated chitin
LPMO / CBP21 (AA10) or AA11	Lytic polysaccharide monooxygenase; EC 1.14.99.53	Serratia marcescens (CBP21) / fungal (AA11)	RG1	CBP21 = BBa in some iGEM projects	2BEM (CBP21)	~20 kDa	AA10/AA11 mono-copper active site	boosts chitinase rate	-	crystalline chitin

Behaviour, conditions & notes

Enzyme	Product / mode	Processive?	pH opt	Temp opt / stability	Metal	His-tag proven	Host expressed	Reaction-order role	Fusion / project note	Refs
SmChiA (WT)	Diacetylchitobiose (GlcNAc)2 from reducing end; even-numbered oligomers dominate	Yes (processive)	~6.0	25-37 C	None	Yes	E. coli (periplasm)	Hydrolyse first	Reference chitinase; multidomain = larger fusion; product is mostly DP2 so pair with a DP2-active COD	S1,S2,S3,S8
SmChiB	(GlcNAc)2 from non-reducing end	Yes (processive)	~6-8	25-37 C	None	Yes	E. coli	Hydrolyse first	Complementary chain polarity to ChiA; useful in a cocktail/synergy control	S2,S8
SmChiA F232W/F396W	As WT, faster on crystalline chitin	Yes (higher processivity)	~6	25 C (assayed)	None	Yes	E. coli	Hydrolyse first	Best if feedstock stays highly crystalline after pre-treatment	S4,S5
ChiA (strain B4A)	chito-oligomers	Yes	7.0	50 C; stable 90 C/60 min; pH 5-8	None	Not stated	native	Hydrolyse first	Thermostable anchor - pairs cleanly at pH 7 / ~50 C with Bacillus or Mucor CDAs	S6
Acid chitinase (chitobiosidase)	Primarily chitobiosidase -> (GlcNAc)2; some endo + GlcNAcase	Partly	~2.8 (very acidic)	50 C	None	Yes (Pichia)	Pichia pastoris	Hydrolyse first	Lowest-pH fungal chitinase known - attractive if the waste stream is acidic; fungal (non-Serratia) biosafety story	S12
Bacillus / Streptomyces GH18 chitinase	chito-oligomers (endo or exo depending on enzyme)	varies	5-8	40-60 C	None	commonly	E. coli / B. subtilis	Hydrolyse first	Cleaner RG1 substitute for Serratia; B. subtilis-native options ease secretion in that chassis	S8
Chitosanase A / B (deacetylate-first route)	GlcN dimer/trimer + higher GlcN oligomers; endo-splitting	No	5.0	50-55 C	None	Not stated	native (fungal)	Depolymerise last (deacetylate-first route)	Prefers deacetylated substrate - the reverse of GH18, so no -1 conflict. Use when a CDA deacetylates the polymer first	S11
ChiC1 (endochitinase C)	endo cuts internally -> range: (GlcNAc)2 main + GlcNAc, (GlcNAc)3, (GlcNAc)4, (GlcNAc)5 -> supplies DP3+	NO (non-processive)	~6-8	37-50 C	Ca2+ (structural site only; GH18 catalysis is metal-free - not a required buffer additive)	Yes	E. coli	Hydrolyse first (endo route) -> feeds PdaC's DP3+ need	The evidence-backed way to get DP3+ oligomers (Horn 2006); CBM12 boosts crystalline-chitin activity	S136,S137, S138,S116, S141
ChiC2 (catalytic domain only)	endo -> DP2-5 range (DP3+ present)	NO	~6-8	37 C	Ca2+ (structural site only; GH18 catalysis is metal-free - not a required buffer additive)	Likely	E. coli	Hydrolyse first (endo route)	Smaller than ChiC1 (drops CBM12) -> better fusion partner, but weaker on crystalline chitin; use amorphous/pre-treated feed	S116,S138
PpChi	Needs partially deacetylated substrate; does not hydrolyse hexa-acetyl-chitohexaose. Produces defined paCOS: DDA (GlcN-GlcN-GlcNAc, 2 amines) + DDDA (GlcN-GlcN-GlcN-GlcNAc, 3 amines)	endo-type	Broad	Broad temp (stable, long shelf life)	None (GH18 substrate-assisted; no metal cofactor)	Yes	E. coli (codon-optimised)	Deacetylate-first (needs DA~48% feed) - not a hydrolyse-first 'Chitinase A' replacement	Not Chitinase A and inverts the order (needs partial deacetylation first). But makes defined multi-amine paCOS directly from shells; very stable; no metal. Best used as a single enzyme after partial (chemical) deacetylation	S72,S175
LPMO / CBP21 (AA10) or AA11	Oxidatively cleaves crystalline chitin -> new chain ends -> boosts oligomer release (6x with PpChi)	N/a (oxidative)	Broad	Broad	Cu (mono-copper active site)	Yes	E. coli	Auxiliary (add to any chitinase)	Not standalone - a booster. Needs O2 + reductant (e.g. ascorbate). Pairs with any construct to raise yield on crystalline feed	S8,S73

Deacetylases

Identity, structure & kinetics

Deacetylase candidates (remove N-acetyl -> free amines for Pt chelation)

Amines per chain drives chelation. CODs = mono/positional (defined but few amines). Broad CDAs / multiple-chain enzymes = more amines, less defined. All CE4 enzymes are metalloenzymes: keep a divalent metal in the reactor buffer and avoid EDTA.

Enzyme	Class / EC	Source organism	RG	Accession (UniProt / GenBank)	PDB	Size	Domain architecture	kcat	Km	Substrate (kinetics)
VcCDA / Cod	COD; EC 3.5.1.105	Vibrio cholerae O1	RG2	UniProt Q9KSH6; GenBank AAF94439.1 / NP_230925.1 (VC1280)	4NYU,4NZ1,4NZ3,4NY2,4OUI	~46 kDa	CE4 catalytic domain + 2 CBM12 (bulky)	Not reported	Not reported	(GlcNAc)2-6
Vp-COD / VpCDA	COD; EC 3.5.1.105	Vibrio parahaemolyticus KN1699	RG2	UniProt A6P4T5; GenBank BAF65669.1	(1.35 A struct.)	~46 kDa	1 polysaccharide-deacetylase domain (PDD) + 2 carbohydrate-binding domains (CBD)	Not reported	Not reported	(GlcNAc)2-3
VhCOD	COD; EC 3.5.1.105	Vibrio campbellii (harveyi)	RG1/2	see ref (recent structures)	6 WT structures (2024-25)	~46 kDa	CE4 catalytic domain + 2 CBM12	Michaelis-Menten (values in ref)	see ref	(GlcNAc)2 (also DP3, lower)
NodB	COD; EC 3.5.1.-	Rhizobium sp. GRH2 / Sinorhizobium meliloti	RG1	gene nodB (R. meliloti; John et al. 1993 PNAS)	-	~25 kDa	Small CE4 / NodB-homology domain (compact)	Not reported	Not reported	(GlcNAc)2-6
ArCE4A	CDA; EC 3.5.1.41	Arthrobacter sp. AW19M34-1 (marine)	RG1	ENA LT630322; UniProt A0A2C8C1T7; PDB 5LGC/5LFZ; 246 aa	5LGC, 5LFZ	246 aa; ~27 kDa	Single CE4 catalytic domain (no accessory CBM) - ideal fusion partner	see ref (activity rises with DP)	Not reported	COS DP2-6 (assayed); crystal with (GlcNAc)2
ClCDA	CDA; EC 3.5.1.41	Colletotrichum lindemuthianum	RG1 (plant pathogen)	UniProt Q6DWK3; GenBank AAT68493.1	(structure solved)	~25 kDa (catalytic)	Single (a/b)7-barrel CE4 catalytic domain, 1 Zn2+	27.1 s^-1	2.55 mM	penta-N-acetyl-chitopentaose
AnCDA	CDA; EC 3.5.1.41	Aspergillus nidulans fgsc A4	RG1	GenBank EAA66447.1	(broad-spec. struct.)	~28 kDa	CE4 catalytic domain (5 motifs)	Not reported	Not reported	COS DP2-6
BaCDA	CDA; EC 3.5.1.41	Bacillus aryabhattai B8W22 / TCI-16	RG1	see ref (cloned)	homology model (5lfz template)	~28-30 kDa	CE4 catalytic domain	3.27x10^4 s^-1 (units in ref are unusual - treat with caution)	see ref (EGC/COS)	ethylene-glycol chitin (EGC) & COS
Mucor rouxii CDA	CDA; EC 3.5.1.41	Mucor rouxii	RG1	cDNA cloned (see ref)	-	~75 kDa (glycoprotein)	CE4 catalytic domain (glycosylated)	Not reported	Not reported	Chitin oligomers / glycol chitin
Penicillium oxalicum CDA	CDA; EC 3.5.1.41	Penicillium oxalicum SAEM-51	RG1	see ref	-	53 kDa	CE4 catalytic domain	Not reported	Not reported	glycol chitin, DP>4 oligomers
Nitratireductor CDA	CDA; EC 3.5.1.41	Nitratireductor aquimarinus MCDA3-3 (marine)	RG1	see ref	-	~30-35 kDa	CE4 catalytic domain	Not reported	Not reported	Insoluble chitin
EsCDA-9k	CDA; EC 3.5.1.41	Euphausia superba (Antarctic krill)	RG1	see ref (1068 bp cDNA)	homology model	355 aa; 45 kDa	CE4 catalytic domain (animal)	Not reported	Not reported	Chitin / COS
BsPdaC (CE4 domain)	CDA-like; CE4	Bacillus subtilis	RG1	gene pdaC (formerly yjeA)	CE4 domain 1.54 A	full multidomain; use CE4 domain only	Membrane-bound multidomain; C-term CE4 catalytic domain	0.24 s^-1 (on GlcNAc4)	12.3 mM (on GlcNAc4)	COS (min substrate GlcNAc3)
BsPdaA	Deacetylase; CE4	Bacillus subtilis	RG1	gene pdaA	-	~26 kDa (truncated)	CE4 domain (signal removed)	Not reported	Not reported	MurNAc / chitin hexamer
Dac / TkCDA	Deacetylase; EC 3.5.1.136; LmbE protein family (not CE4/NodB family)	Thermococcus kodakarensis KOD1	RG1	UniProt Q6F4N1; gene TK1764 (Dac)	-	~26 kDa	LmbE-family de-N-acetylase domain (single domain, archaeal) - not CE4	Not reported	Not reported	N,N'-diacetylchitobiose (GlcNAc)2

Specificity, conditions & notes

Enzyme	Positional specificity / amines per chain	Substrate DP range	Insoluble chitin?	pH opt	Temp opt / stability	Metal	His-tag proven	Host expressed	Reaction-order role	Fusion / project note	Refs
VcCDA / Cod	mono: penultimate GlcNAc from non-reducing end (1 amine); broad DP2-6	DP2-DP6	Slight only	~7-8	~30 C	Zn2+	Yes (COD-6His)	E. coli	Deacetylate after hydrolysis	Best-characterised COD, many PDBs, directed-evolution libraries exist. RG2 source -> use synthetic gene. Bulky (2x CBM12) for a fusion	S20,S21,S24,S25,S39,S84
Vp-COD / VpCDA	mono on DP2 (and DP3, lower); narrow	DP2-DP3	No	~7-8	~30-37 C	Zn2+ (CE4)	Yes	E. coli	Deacetylate after hydrolysis	Very high-res structure; CBD deletion barely lowers activity (can trim for a smaller fusion). RG2 source	S23,S39
VhCOD	mono: (GlcNAc)2 -> GlcNAc-GlcN (1 amine)	DP2 (DP3 weak)	No	~8.5 (borate assay)	30 C	Zn2+ (His-His-Asp)	Yes	E. coli	Deacetylate after hydrolysis	Newest structural data on the catalytic cycle; narrow DP2 focus matches ChiA's (GlcNAc)2 output	S22
NodB	mono: exclusively the non-reducing terminal GlcNAc; not active on GlcNAc1	DP2-DP6	No	7-8	30 C	divalent (CE4)	Yes (Trx-fusion, high yield)	E. coli	Deacetylate after hydrolysis	Stack with VcCDA (Hamer et al.) -> defined DI-deacetylated non-reducing end (2 amines). Compact, folds well, RG1	S46,S49,S50,S52,S89
ArCE4A	Broad, multiple-chain; internal sugars deacetylated first; leaves reducing-end acetyl -> pattern [Dn-1A] (like PdaC)	DP5 > DP6 ~ DP4 > DP3 >> DP2 (strongly prefers long oligomers; weak on DP2)	Low on crystalline (WT); active on chitosan DA64% & insoluble chitin at reduced rate	~7-8	~30-40 C	Ni2+ (native; CE4 His-His-Asp metal site)	Yes	E. coli	Flexible (either order)	Strongly prefers DP4-5, weak on DP2 -> does not match ChiA's DP2 output (same/worse DP-gap as PdaC). Best paired with a long-oligomer chitinase (ChiC), not ChiA. Single 246 aa domain still good for folding. Engineered Mut-2-8 improves crystalline activity	S56,S57,S58,S62,S65,S190,S191
ClCDA	Recognises 4 subsites (-2,-1,0,+1); deacetylates subsite 0; multiple-chain (random) on polymer	DP>=2 (better longer)	Weak	~7-8.5	~50 C	Zn2+	Yes (His6; slight kcat drop)	E. coli (secreted)	Flexible (either order)	Classic, hard kinetics available; multi-deacetylation raises amine count. Plant-pathogen source = minor flag; synthesise gene	S11,S12,S14,S39,S72
AnCDA	Broad specificity -> multiple amines	DP2-DP6	Moderate	~7	~50 C	Co2+ (cobalt, not zinc) - CE4	Yes	E. coli	Flexible (either order)	Broad-specificity fungal CDA; good amine density; RG1	S88,S64
BaCDA	Deacetylates EGC & COS -> chitosan	polymer + COS	EGC (soluble)	7.0	30 C; thermostable (87-94% after 50 C/24 h)	Mg2+ activated	Yes	E. coli Rosetta pLysS	Flexible (either order)	Halotolerant + thermostable + RG1 Bacillus - strong reactor/biosafety profile; verify the odd published units	S10,S11,S13,S60,S82
Mucor rouxii CDA	Multiple amines on oligomers (not on insoluble chitin)	oligomers	No	7.0	50 C	Co2+ activated	Historically native	native / E. coli attempts	Flexible (either order)	First CDA discovered; pH7/50C matches B4A ChiA well. Glycosylation may complicate bacterial expression	S74,S75,S82
Penicillium oxalicum CDA	Deacetylates DP>4 well -> several amines	DP>4	glycol chitin	9.0 (alkaline)	50 C; t1/2 693 min	Cu2+/Co2+	Not stated	native	Flexible (either order)	Alkaline optimum - only if reactor runs basic; long half-life is attractive	S76
Nitratireductor CDA	Deacetylates insoluble chitin directly -> chitosan (multiple amines)	polymer (insoluble)	Yes (rare)	8.0	30 C	divalent (CE4)	Yes	E. coli	Deacetylate first	Rare ability to act on insoluble chitin - enables the CDA-first route without pre-solubilisation; RG1 marine	S81
EsCDA-9k	Deacetylation -> chitosan	polymer + COS	Some	8.0 (Tris)	40 C	Co/Fe/Ca/Na enhance; Zn/Ba/Mg/EDTA inhibit	Yes	E. coli (soluble)	Flexible (either order)	Crustacean-derived (thematically fits shellfish-waste story); F18G mutant = 1.6x activity; note EDTA/Zn inhibit this one	S18,S75

Enzyme	Positional specificity / amines per chain	Substrate DP range	Insoluble chitin?	pH opt	Temp opt / stability	Metal	His-tag proven	Host expressed	Reaction-order role	Fusion / project note	Refs
BsPdaC (CE4 domain)	Multiple-chain: deacetylates all but the reducing-end GlcNAc -> highest amine density here; activity rises with DP	DP>=3	No	~7	37 C	Mn2+	Yes (CE4 domain)	E. coli	Deacetylate after hydrolysis	Chassis-native to B. subtilis; express the isolated CE4 domain, not the membrane protein. Max amines but heterogeneous product	S66,S67,S68,S70,S72
BsPdaA	Primarily MurNAc deacetylase; acts on (GlcNAc)6 -> some amines	DP6 tested	No	5.5-10 range tested	37 C	metal (CE4)	Yes (His, truncated)	E. coli	Flexible	Chassis-native; weaker/less-specific chitin activity than PdaC - keep as backup	S71
Dac / TkCDA	mono: deacetylates the non-reducing GlcNAc of chitobiose -> GlcN-GlcNAc (1 amine); also acts on GlcNAc monomer	DP2 (and GlcNAc1)	No	Near-neutral	High (hyperthermophile - very thermostable)	LmbE family (metal use differs from CE4 - verify; not the CE4 Mn2+/Zn2+ pattern)	Yes	E. coli	Deacetylate after hydrolysis	Matches ChiA's native DP2 exactly -> no DP3+ tuning. iGEM: novel (no registry part). Hyperthermophile: verify activity at 37-45 C. Stacks with COD (opposite end) for GlcN-GlcN	S118,S121,S124,S186,S187,S188

Secondary Requirements

Secondary requirements - fusion, reactor & biosafety fit

Qualitative fit for the fusion context. '++' strong / '+' ok / '~' conditional / '-' weak. Use alongside the kinetic detail on the enzyme sheets.

Enzyme	Role	Single-domain (fusion-friendly)	His-tag tolerant	Metal needed (buffer)	pH match to ChiA (pH6-7)	Thermostable	Expression proven (E.coli/B.sub)	Biosafety (source)	Amines / chelation potential
SmChiA (WT)	Chitinase	- (3 domains)	++	None	++	-	++ E. coli	+ RG1 (opportunistic)	N/a (hydrolysis)
ChiA B4A	Chitinase	- (multidomain)	~	None	++ (pH7)	++ (90C/60min)	~ native	+ RG1	N/a
Chitosanase (Mucor)	Depolymerase (deacetylate-first route)	+	~	None	~ (pH5)	+	~ fungal	++ RG1	N/a (releases GlcN oligomers)
VcCDA / Cod	Deacetylase (COD)	- (2x CBM12)	++	Zn2+	+ (pH7-8)	-	++ E. coli	- RG2 pathogen	+ (1 amine, defined)
Vp-COD	Deacetylase (COD)	~ (CBDs trimmable)	+	Zn2+	+	~	+ E. coli	- RG2 pathogen	~ (1 amine, DP2-3 only)
NodB	Deacetylase (COD)	++ (compact)	++	divalent	+ (pH7-8)	~	++ E. coli (Trx)	++ RG1	+ (1 amine; 2 when stacked w/ VcCDA)
ArCE4A	Deacetylase (CDA)	++ (246 aa, 1 domain)	++	divalent	+ (pH7-8)	~	++ E. coli	++ RG1 marine	++ (multiple, internal)
ClCDA	Deacetylase (CDA)	+ (1 catalytic domain)	+ (slight kcat drop)	Zn2+	+	+ (50C)	++ E. coli	+ RG1 (plant path.)	++ (multi-deacetylation)
BaCDA	Deacetylase (CDA)	+	++	Mg2+	++ (pH7)	++ (50C/24h)	++ E. coli Rosetta	++ RG1 Bacillus	+ (polymer+COS)
Mucor rouxii CDA	Deacetylase (CDA)	+ (glycoprotein)	~	Co2+	++ (pH7)	+ (50C)	~ (glycosylation)	++ RG1	++ (oligomers)
Nitratireductor CDA	Deacetylase (CDA)	+	+	divalent	~ (pH8)	~	+ E. coli	++ RG1 marine	++ (acts on insoluble chitin)
EsCDA-9k	Deacetylase (CDA)	+	++	Co/Ca (EDTA/Zn inhibit)	~ (pH8)	~	++ E. coli soluble	++ RG1 (krill)	+ (thematic: shellfish)
BsPdaC CE4 domain	Deacetylase (CDA-like)	++ (isolated domain)	++	Mn2+	+ (pH7)	~	++ E. coli / native B.sub	++ RG1 chassis-native	++ (all but reducing end = most amines)

Constructs (all feasible)

All feasible constructs to satisfy the project (chitin -> deacetylated chelating agent -> chelate cisplatin -> filter)

Grouped by construct type. The project's stated deliverable is a two-part fusion (Chitinase A + one deacetylase) = rows F1-F7. Everything below that relaxes the 'single fusion' premise and should be labelled as such on the wiki. No chitinase-deacetylase fusion was found in the literature, so the core fusion is novel.

A. two-part fusion (Chitinase A + one deacetylase = the project's stated deliverable)

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
F1	Two-part fusion	ChiA (S. marcescens)	VcCDA / COD (V. cholerae)	1 (reducing GlcNAc of DP2)	ChiA's native DP2 - matched	E. coli (COD proven); B. subtilis	N-ChiA-(GGGGS)2/3-COD-C and N-COD-linker-ChiA-C; also test rigid (EAAAK)3; His on free terminus + untagged control	COD proven & improved (Darmstadt'17; Stuttgart'18 BBa_K2848001). ChiA parts exist but expression mostly failed	Fusion is novel (no ChiA-deacetylase fusion exists)	pH 6-7; ~37 C; +Zn (COD); no EDTA	Medium - COD easy, ChiA hard	ChiA expression is the bottleneck; 1 amine may be a weak (monodentate) Pt binder	S20,S41,S45,S180
F2	Two-part fusion	ChiA (S. marcescens)	Dac / TkCDA (T. kodakarensis)	1 (non-reducing GlcNAc of DP2)	DP2 matched; natural ChiA->Dac pathway exists in T. kodakarensis	E. coli	fuse; test both orientations + flexible/rigid linker	Dac novel (no part); ChiA parts poor	Fusion + Dac both novel -> Best-Part potential	pH ~7; Dac native 70 C (verify 37-45 C activity); LmbE metal	Medium	Dac hyperthermophile - may be sluggish at mesophilic temp; ChiA expression	S52,S186, S188
F3	Two-part fusion	ChiA (S. marcescens)	NodB (S. meliloti)	1 (non-reducing terminal)	DP2-6	E. coli	fuse	NodB proven (BBa_K2380041) but forms inclusion bodies / needs refolding, pH 9. ChiA poor	Fusion novel	NodB pH 8-9 vs ChiA pH 6 = mismatch; divalent metal	Low-med	NodB inclusion bodies + pH clash with ChiA	S45,S183, S184
F4	Two-part fusion	ChiA (S. marcescens)	PdaC CE4 domain (B. subtilis)	Multi (all but reducing end)	Needs DP3+ but ChiA gives DP2 (gap)	B. subtilis (PdaC native)	N-ChiA-linker-PdaC(CE4 domain only)-C	PdaC novel; ChiA poor	Fusion + application novel	pH 7; ~37 C; +Mn2+; no EDTA	Low-med	DP3+ gap; PdaC slow (kcat 0.24/s); ChiA hard	S66,S67,S68
F5	Two-part fusion	ChiA (S. marcescens)	ArCE4A (Arthrobacter)	Multi (Dn-1A pattern) - but see mismatch	ArCE4A prefers DP4-5, weak on DP2 -> same DP-gap as F4 (mismatch with ChiA)	E. coli	ArCE4A is a single 246 aa domain -> best fusion geometry; test orientations	ArCE4A novel; ChiA poor	Fusion novel	pH 7-8; +Ni2+/divalent; no EDTA	Low-med	ArCE4A is weak on ChiA's DP2 product (DP5>>DP2). Single-domain folding is nice but substrate clashes with ChiA. Pair ArCE4A with ChiC (long oligomers), not ChiA	S56,S62,S65
F6	Two-part fusion	ChiA (S. marcescens)	ClCDA (Colletotrichum)	Multi	DP2+	E. coli (periplasm / MBP-fusion)	fuse; express as MBP-fusion or periplasmic to rescue solubility (fungal CDA precedent)	ClCDA not in registry; ChiA poor	Fusion novel	pH 7-8.5; +Zn	Low-med	Fungal CDA hard to express (use MBP/periplasm); plant-pathogen source	S11,S174
F7	Two-part fusion	ChiA (S. marcescens)	BaCDA (B. aryabhattai)	Multi / polymer	polymer + COS	E. coli / B. subtilis	fuse	BaCDA novel; ChiA poor	Fusion novel; RG1 Bacillus source	pH 7; thermostable; +Mg2+	Medium	BaCDA characterised on soluble EGC - confirm on the product; ChiA hard	S10,S13,S60

B. two-part fusion using ChiC (endo-chitinase - not literally 'Chitinase A', but solves the DP3+ supply)

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
F8	Two-part fusion (ChiC)	ChiC1/ChiC2 (Sm endo)	PdaC CE4 domain	Multi	ChiC endo range supplies DP3+	B. subtilis	ChiC2 (compact, no CBM) fusion	ChiC not in registry; PdaC novel	Novel; solves DP gap	pH 7; +Mn2+	Medium	ChiC still DP2-dominant (quantify DP3+ yield); deviates from 'ChiA'	S116,S138,S66
F9	Two-part fusion (ChiC)	ChiC2 (compact)	ArCE4A (single domain)	Multi	ChiC supplies DP3+	E. coli	Two compact single domains -> cleanest fusion	both novel	Novel	pH 7-8; divalent	Medium	Deviates from 'ChiA'; confirm activity on ChiC product mix	S116,S62

C. two-part fusion using an alternative, robust chitinase (defined product, crustacean-tested)

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
F11	Single enzyme (deacetylate-first) - not a two-part fusion	PpChi (Paenibacillus pabuli) - GH18, needs DA~48% feed	none in the enzyme step (PpChi's product is already multi-amine)	2-3 (DDA = 2 amines; DDDA = 3 amines), defined single glycoforms	Needs partially deacetylated chitin (DA~48%) first; doesn't touch fully acetylated chitin	E. coli	No fusion needed. Flow: (chemical/enzymatic partial deacetylation in chitin-prep) -> PpChi cleaves -> defined paCOS	PpChi not in registry (novel)	Novel; defined multi-amine paCOS from crustacean shells	PpChi: NO metal (GH18). Optional LPMO booster: Cu + O2 + reductant. If deacetylation is enzymatic upstream: that CDA needs a divalent metal	Medium-high (robust enzyme) but deviates from the 'ChiA + deacetylase fusion' spec	Not Chitinase A and not hydrolyse-first. Relocates deacetylation into the chitin-prep step. Verify DDA/DDDA yield on the target waste stream (DA varies)	S72,S175

D. fusion + free enzyme / multi-enzyme strain (relaxes 'single fusion' -> reaches 2+ amines). label these clearly - not a two-part fusion.

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
M1	Multi-enzyme strain	ChiA	Dac + COD (opposite ends)	2 (GlcN-GlcN, bidentate)	DP2	E. coli	Not a single fusion: co-express 3 enzymes (or ChiA-fusion + free Dac + free COD)	COD proven; Dac novel; ChiA poor	Novel product (fully deacetylated chitobiose)	pH 7; +Zn/metal	Medium	Confirm each deacetylase accepts the other's product; not a 2-part fusion	S53,S187, S20
M2	Multi-enzyme strain	ChiA	NodB + COD	2 (defined di-deacetylation)	DP3+	E. coli	co-express (Darmstadt orthogonal-induction precedent)	NodB+COD proven together (Darmstadt'17); ChiA poor	Application novel; enzyme combo already done by Darmstadt	NodB pH8-9 / COD pH7; metals	Medium	Darmstadt already did NodB+COD -> novelty here is the application; not a fusion	S45,S183, S48
M3	Fusion + free enzyme	ChiC-PdaC fusion	+ free Dac (DP2 mop-up)	Multi + DP2 rescued	ChiC range	B. subtilis	ChiC-PdaC fusion plus free Dac	mixed	Novel	pH 7; +Mn2+/metal	Medium-low	4 activities; high complexity	S116,S66, S53

E. deacetylate-first - ChiA is excluded (GH18 can't cut deacetylated chitosan); uses a chitosanase instead

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
B1	Deacetylate-first	Mucor chitosanase (cuts after deacetylation)	Nitratireductor CDA (acts on insoluble chitin)	Multi (highly deacetylated GlcN oligomers)	polymer -> oligomer	E. coli / native	Not a ChiA fusion: CDA on polymer, then chitosanase	neither in registry	Novel	CDA pH8/30C then chitosanase pH5/50C (two-pot)	Low-med	Two pH optima -> likely two-pot, not one fusion; no ChiA	S81,S11
B2	Deacetylate-first	Mucor chitosanase	BaCDA (soluble/EGC)	Multi	polymer -> oligomer	E. coli	two-step	BaCDA/chitosanase novel	Novel	pH7 / 50C for both	Medium	BaCDA on EGC - confirm on real chitin	S10,S11

F. polymer chelator ('similarly-effective chelating agent' - deacetylate only, no cutting enzyme; product is chitosan polymer, not an oligosaccharide)

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
P1	Polymer chelator (no hydrolase)	- none -	Nitratireductor CDA (insoluble chitin) -> chitosan	many (chitosan polymer amines)	polymer stays polymer	E. coli / native	Single deacetylase, no fusion	Nitratireductor novel	Simplest enzymology (one enzyme)	pH 8 / 30 C; metal	Medium	chitosan insoluble near neutral pH -> dosing/filtration harder; but a valid chelating agent	S81,S93
P2	Polymer chelator	- none -	Any CDA on colloidal/pre-treated chitin -> chitosan	many	polymer	E. coli	one enzyme	varies	Simple	pH per CDA	Medium	Needs pre-solubilised chitin; insolubility caveat	S93,S96

G. auxiliary (add to any construct above to boost oligomer release from crystalline chitin)

ID	Construct type	Hydrolase	Deacetylase(s)	Amines/chain (chelation)	DP handling	Chassis fit	Fusion design (orientation + linker)	iGEM Registry precedent	Novelty	Conditions (pH/temp/metal)	Feasibility / risk	Watch-outs	Refs
Aux	Auxiliary booster	LPMO: CBP21/AA10 (Sm) or AA11 (fungal)	Pairs with any chitinase	N/a	Oxidatively cleaves crystalline chitin -> new chain ends	E. coli	co-express or add separately	CBP21 used in some iGEM projects	boosts yield	O2 + reductant (ascorbate)	High value	Needs O2/reductant; adds complexity	S8,S73

Fusion Design & Expression

Fusion design & expression - how to actually build a two-part fusion

Parameter	Recommendation	Why / evidence	Refs
Orientation	Test both: N-ChiA-linker-DAC-C and N-DAC-linker-ChiA-C	Orientation changes distance/geometry between domains and which terminus is free for a tag; empirically it can swing activity, so it must be tested, not assumed	S170,S172
Linker - flexible	(GGGGS)2 or (GGGGS)3	Flexible GS linkers improve folding and let domains move/orient independently; classic (GGGGS)3 used for scFv; good where substrate hand-off is wanted between the two active sites	S66lnk,S170,S66exp
Linker - rigid	(EAAAK)2-3 (alpha-helical)	Rigid helical linkers separate the two domains so each folds/functions independently, and improve thermostability (shown for xylanase-mannanase, beta-glucanase fusions)	S170,S172
Linker - best practice	Screen a small library (flexible / rigid / combined e.g. FFFRR)	The optimum is often neither fully rigid nor fully flexible; a mixed linker gave peak activity in a PhoC-GFP linker-library study	S170
His-tag placement	His6 on the free terminus; always run an untagged control	Terminal extensions measurably lowered ClCDA kcat; one terminus is already spent on the linker, so tag the other and check the tag isn't costing activity	S12
Secretion signal	ompA / Sec (E. coli) or AmyQ/SacB (B. subtilis)	Substrate (chitin) is extracellular, so secretion helps; the (retracted) chitinase-protease fusion used ompA for extracellular export	S173
Difficult deacetylases	Express as MBP-fusion or in the periplasm	Fungal/inclusion-body-prone CDAs (ClCDA, NodB) are hard to express; a fungal CDA was rescued as an MBP-fusion in E. coli periplasm to get soluble, active enzyme	S174,S184
Chassis - B. subtilis	GRAS; strong native secretion; PdaC is native	Best for secreted multidomain enzymes and for PdaC-based fusions; fewer iGEM parts/tools than E. coli	S66,S67
Chassis - E. coli	Easiest; most iGEM parts; COD/NodB precedents exist	Fastest to build and the deacetylases with iGEM history (COD, NodB) were done here; weaker secretion, endotoxin	S45
Auxiliary enzyme	Add an LPMO (CBP21/AA10 or AA11) + reductant	Oxidatively opens crystalline chitin, giving chitinases more chain ends - 6x more oligosaccharide release measured with PpChi on lobster shell	S73,S8
Metal in buffer	Match to the deacetylase (Zn for CE4/COD; Mn2+ for PdaC; none for ChiA), and remove before chelation	Deacetylases are metalloenzymes; but any residual metal competes with Pt for the amines, so buffer-exchange the product before the waste stream	S82,S67
Validation set	(1) SDS-PAGE expression; (2) HPLC/MS product DP + amine profile; (3) acetate-release assay; (4) surrogate-metal (Pd/Cu) binding	Proves the fusion expresses, cuts to the right DP, deacetylates, and that the product binds a Pt-like metal - the minimum to call the construct demonstrated	S67,S73

iGEM Registry Precedent

iGEM registry precedent - what already exists, and whether it actually worked

Key point for supervisors: 'already in the Registry' is an asset (de-risked, build on it), not a reason to avoid it. Novelty here is the cisplatin-chelation application + the fusion, not the individual enzymes. The real feasibility signal is whether a part expressed and worked (below).

Enzyme	Part(s) / team	Status - did it work?	Implication	Refs
ChiA (S. marcescens)	BBa_K1913000/002 (Wageningen 2016)	Expression failed - no significant band; chitin assay gave no result	Biggest feasibility risk. Learn from their conditions; consider the 2025 working part or a Bacillus/PpChi chitinase	S180
ChiA (S. marcescens)	BBa_K1201000 (2013, Dundee gift)	No activity alone; positive only with ChiA+ChiB+ChiC together	ChiA may need its partner chitinases or an LPMO to show activity	S181
ChiA (S. marcescens gei)	'Smchi' (Uprize-I 2025)	Expressed (Coomassie-verified), with 3 Bacillus chitinases	A recent working expression exists - use their protocol/part as a starting point	S182
VcCDA / COD	BBa_K2380xxx (Darmstadt'17); BBa_K2848001 improved (Stuttgart'18)	Works, characterised, and already improved once	De-risked. Build on the improved COD rather than starting over	S45,S185
NodB (S. meliloti)	BBa_K2380041 (Darmstadt 2017)	Works but forms inclusion bodies (needs refolding); pH 9 optimum	Usable but needs MBP-fusion/periplasm rescue; pH clashes with ChiA	S184
NodC (chitin synthase)	BBa_K2380000/002 (Darmstadt 2017)	Works - makes chitin pentamers in E. coli	Not needed when starting from real chitin, but available	S39
Linker design	BBa_K4990005 (2023)	Reference part: GGGGS/EAAAK linker guidance	Use as a template for the linker screen	S172
Dac / TkCDA	none	Not in Registry	Novel - Best-Part opportunity if characterised	S186
PdaC / ArCE4A / ClCDA / BaCDA / ChiC / PpChi / Nitratireductor CDA	none	Not in Registry	All novel to iGEM - opportunity + must characterise from scratch	-

Decision Path

Decision path - two experiments decide the construct

The construct list looks big, but only two experiments actually gate the choice. Run them first; each result prunes the tree.

Where this sits IN the project (all three stages)

Stage	What happens	Decision it forces (affects enzyme choice)		
1. Upstream	Shellfish waste -> chitin (chemical/enzymatic protocol)	Key fork: leave chitin fully acetylated (feeds a ChiA fusion) OR partially deacetylate to DA~48% (opens the single-enzyme PpChi route, F11). The prep protocol already has a chemical deacetylation step, so this is a dial, not a new step.		
2. The cell (fusion)	Engineered B. subtilis/E. coli makes the enzyme(s)	Governed by gate 1 (does the chitinase work?) and gate 2 (how many amines?) below		
3. Downstream	Chelate cisplatin in the waste, then filter	Product DP sets the filtration method: soluble oligomer -> adsorbent/ultrafiltration; chitosan polymer -> easier to filter but insoluble near neutral pH. And all reactor metals must be removed first (they compete with Pt for amines).		

Gate 1 - expression (the critical path - do this first)

Question: does the chitinase express and cut chitin in the chosen chassis? (Test: SDS-PAGE for expression + colloidal-chitin clearing / chitin-azure assay for activity.) Why first: Sm ChiA failed to express for two prior iGEM teams and was active only as a ChiA+ChiB+ChiC trio for another - so this is the single most likely thing to sink the project.

If...	Then...	Construct / action	Refs	
Yes - it expresses & cuts	Go to gate 2 keeping ChiA	Proceed with ChiA-based fusions	S180-182	
NO - poor/no expression	Switch the chitinase before anything else, then re-test Gate 1	(a) use the 2025 Uprize-I working ChiA part/protocol; (b) a Bacillus chitinase (iGEM-expressed); (c) co-express ChiA+ChiB+ChiC (the trio that worked in 2013); (d) add an LPMO/CBP21 booster + reductant	S180,S181,S182,S73	

Gate 2 - chelation stoichiometry (cisplatin-free surrogate experiment)

Question: how many free amines per chain does cisplatin capture actually need? (Test: make a 0/1/2/many-amine ladder, bind a surrogate metal - Pd(II) as the isostructural Pt(II) analogue, or Cu(II) as a cheap screen - and run a Job plot for stoichiometry + adsorption isotherm for capacity. No cisplatin needed. See 'The two experiments' below.)

If the answer is...	Recommended construct	Product (amines)	Why	Refs
1 amine is enough	F1: ChiA + COD (true two-part fusion)	GlcNAc-GlcN (1 amine)	COD genuinely acts on DP2 (ChiA's product) and is the only deacetylase with a working iGEM record. Simplest thing that matches the 'ChiA + deacetylase fusion' spec.	S20,S45
2 amines needed	M1: ChiA + Dac + COD (multi-enzyme strain)	GlcN-GlcN (2 amines, bidentate)	Dac (non-reducing) + COD (reducing) fully deacetylate chitobiose - no DP3+ needed. 3 enzymes, so label it a multi-enzyme strain, not a single fusion.	S53,S187
3+ amines on one oligomer	F8/F9: ChiC + PdaC or ArCE4A	DP3-5 multi-amine	Needs long oligomers, so processive ChiA must be dropped for endo-ChiC. PdaC/ArCE4A only work on DP3+.	S116,S66
2-3 amines, simplest route	F11: PpChi (single enzyme, deacetylate-first)	DDA/DDDA (2-3 amines, defined)	If the upstream step can partially deacetylate to DA~48%, PpChi alone makes defined multi-amine paCOS from shells. But this is not a ChiA fusion (changes the headline).	S72,S175

Gate 3 - deliverable constraint

Question: must the deliverable be a single fusion protein, or is a multi-enzyme strain acceptable? (This is a project-scope decision for the team + supervisors, not an experiment.)

If...	Then available	Excluded		
Single fusion required (as written in the spec)	F1 (1 amine) or F8/F9 (multi-amine via ChiC)	M1 (it's a 3-enzyme strain, not one protein)		
Multi-enzyme strain acceptable	Also M1 (GlcN-GlcN, 2 amines) and M2 (NodB+COD, Darmstadt precedent)	-		

Leaf recommendations (where each path lands)

Path (Gate outcomes)	Recommended construct	Product	Why	Flag
ChiA works + 1 amine ok + single fusion	F1 (ChiA + COD)	GlcNAc-GlcN	Simplest true fusion; de-risked deacetylase	Recommended
ChiA works + 2 amines + strain ok	M1 (ChiA + Dac + COD)	GlcN-GlcN	Bidentate chelator; no DP3+ problem	Recommended
ChiA works + 3+ amines	F8 (ChiC + PdaC)	DP3-5 multi-amine	Long oligomers feed a multi-deacetylase	Conditional
Upstream partially deacetylates (DA~48%)	F11 (PpChi single enzyme)	DDA/DDDA (2-3 amines)	Simplest overall; defined product from shells; but not a ChiA fusion	Conditional
ChiA fails Gate 1	Switch chitinase (Bacillus / PpChi / 2025 part), re-enter tree	-	Expression is the gate, not the strategy	Conditional
All fusion routes stall	P1 (CDA -> chitosan polymer)	chitosan (many amines)	Fallback 'similarly-effective chelating agent'; insoluble near neutral pH	Fallback

The two gating experiments (what to actually run)

Experiment	Steps	Read-out / decision	Refs	
1. Expression & activity (Gate 1)	Transform construct into E. coli BL21 or B. subtilis; induce (IPTG/arabinose); SDS-PAGE; activity by colloidal-chitin clearing or chitin-azure assay. Borrow prior iGEM protocols.	Band at expected size + chitin cleavage = pass. Use the 2025 working part's conditions if this fails.	S180,S181,S182	
2. Chelation stoichiometry (Gate 2) - NO cisplatin	(a) Make an amine ladder: 0 / 1 / 2 / many amines (fully-acetylated COS / mono-deacetylated / GlcN-GlcN / chitosan). (b) Confirm amines by TNBS or ninhydrin; degree of deacetylation by potentiometric titration/FTIR. (c) Bind a surrogate metal: Pd(II) (PdCl2, isostructural Pt(II) analogue) or Cu(II) (cheap, colorimetric). (d) Job plot -> stoichiometry; batch adsorption isotherm (Langmuir) -> capacity; UV-vis/itc -> strength. (e) Quantify residual metal by spectrophotometric titration / mip-oes / ICP.	Capacity & stoichiometry vs amine density = the answer to 'how many amines'. A single amine is monodentate (likely weak); >=2 gives the chelate effect.	S200,S201,S202,S203	
Final confirmation (once, later)	Real cisplatin, residual Pt by ICP-MS, at a licensed partner facility (hospital pharmacy / analytical lab) - not the undergrad lab.	Confirms surrogate result transfers to the drug; also a Human-Practices partnership.	S200,S201	

Metal note: whichever divalent metal the deacetylase needs (Zn for COD, Mn for PdaC, Ni for ArCE4A, Cu for LPMO) must be removed by buffer-exchange before the paCOS meets the waste - free metal competes with Pt for the amines (EDTA desorbs metal from chitosan).

Sources

Sources (cited by ID in every 'Refs' cell)

ID	Reference (short)	Type	URL
S1	Sorbotten et al. - natural-substrate HPLC assay; SmChiA/ChiB kcat & Km	Journal (ScienceDirect)	https://www.sciencedirect.com/science/article/abs/pii/S0003269707000036
S2	Comparative studies of chitinase A and B from Serratia marcescens	Journal	https://www.researchgate.net/publication/14444395
S3	UniProt P07254 - Chitinase A, Serratia marcescens	Database (UniProt)	https://www.uniprot.org/uniprot/P07254
S4	Single-molecule imaging of SmChiA F232W/F396W high-activity mutant	Journal (PMC)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7029130/
S5	SmChiA F232W/F396W mechanism - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/31924658/
S6	Emruzi et al. 2020 - kinetic/thermo-inactivation of S. marcescens B4A chitinase	Journal	https://www.bmmj.org/article_244013.html
S8	Vaaje-Kolstad et al. 2013 - chitinolytic machinery of S. marcescens (review)	Journal (FEBS)	https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.12181
S10	Pawaskar et al. 2021 - chitin deacetylase from Bacillus aryabhattai B8W22	Journal (Springer)	https://link.springer.com/article/10.1007/s13205-021-03073-3
S11	BaCDA B8W22 - PMC full text (incl. ClCDA/Mucor comparison)	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC8636556/
S12	Tokuyasu et al. 1999 - recombinant ClCDA in E. coli (His-tag effect on kcat)	Journal (FEBS Lett)	https://febs.onlinelibrary.wiley.com/doi/10.1016/S0014-5793(99)01113-8
S13	BaCDA characterization - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/34917446/
S14	Recombinant CDA production in E. coli - ScienceDirect	Journal	https://www.sciencedirect.com/science/article/pii/S0014579399011138
S18	Cloning & characterization of chitin deacetylase from Euphausia superba (EsCDA-9k)	Journal (mdpi/PMC)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10889134/
S20	Li et al. 2007 - V. cholerae chito-oligosaccharide deacetylase (COD) - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/17884842/
S21	V. cholerae COD - Glycobiology (Oxford)	Journal	https://academic.oup.com/glycob/article/17/12/1377/1988049
S22	Structure & loop dynamics of VhCOD (V. campbellii) - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/40835009/
S23	Structure-function of Vp-COD (V. parahaemolyticus) - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/25479092/
S24	PDB 4OUI - VcCDA with triacetylchitotriose	Database (rcsb)	https://www.rcsb.org/structure/4OUI
S25	Hts assay / directed evolution of VcCDA - Anal. Chem.	Journal (acs)	https://pubs.acs.org/doi/10.1021/acs.analchem.8b02729
S39	CAZy CE4 - accessions for VcCDA (Q9KSH6, AAF94439.1), Vp-COD (A6P4T5), ClCDA, AnCDA	Database (CAZy)	http://www.cazy.org/CE4_structure.html
S46	S. meliloti NodAB/NodH on thiochitooligosaccharides - ResearchGate	Journal	https://www.researchgate.net/publication/11293229
S49	Grifoll-Romero et al. - Chitin deacetylases: structures, specificities, biotech (review)	Journal (PMC)	https://pmc.ncbi.nlm.nih.gov/articles/PMC6415152/
S50	High-yield Rhizobium NodB in E. coli for lco precursors - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/28284052/
S52	John et al. 1993 - Rhizobium NodB is a chitooligosaccharide deacetylase - PMC	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC45716/
S56	Rationally engineered ArCE4 (Mut-2-8) for crystalline chitin - ScienceDirect	Journal	https://www.sciencedirect.com/science/article/abs/pii/S0144861721010249
S57	Engineered ArCE4 - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/34702460/
S58	PDB 5LGC - ArCE4A (T48 deacetylase) with substrate	Database (rcsb)	https://www.rcsb.org/structure/5LGC
S60	Bacillus aryabhattai TCI-16 CDA - Frontiers	Journal	https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.999639/full
S62	Tuveng et al. 2017 - ArCE4A structure & function (246 aa, single domain) - PMC	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC5673215/
S64	Insect chitin deacetylases review (ClCDA/AnCDA/ArCE4A/VcCDA structures) - PMC	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC7953033/
S65	ArCE4A - plos one full text	Journal (plos)	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0187544
S66	Kobayashi et al. 2012 - PdaC from B. subtilis - PubMed	PubMed	https://pubmed.ncbi.nlm.nih.gov/22277649/
S67	PdaC (B. subtilis) - PMC full text	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC3323032/
S68	BsPdaC dual MurNAc/GlcNAc specificity, CE4 domain 1.54 A - ResearchGate	Journal	https://www.researchgate.net/publication/337054972
S70	BsPdaC structure-function - PMC	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC6916482/
S71	PdaA from B. subtilis acts as MurNAc deacetylase in vitro - PMC	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC545626/
S72	ClCDA subsite recognition of chitooligosaccharides - Biochemistry (acs)	Journal	https://pubs.acs.org/doi/10.1021/bi0005355
S74	Purification & characterization of CDA from Rhizopus nigricans - ScienceDirect	Journal	https://www.sciencedirect.com/science/article/abs/pii/S0141022906001451
S75	EsCDA-9k (mdpi) incl. Mucor rouxii pH7/50C optima table	Journal (mdpi)	https://www.mdpi.com/1422-0067/25/4/2075
S76	CDA from Penicillium oxalicum SAEM-51 (pH9, 50C) - ScienceDirect	Journal	https://www.sciencedirect.com/science/article/abs/pii/S0144861711007259
S81	CDA active on insoluble chitin from Nitratireductor aquimarinus - ScienceDirect	Journal	https://www.sciencedirect.com/science/article/abs/pii/S0141813020302063
S82	BaCDA B8W22 metal/pH data - PMC	PMC	https://pmc.ncbi.nlm.nih.gov/articles/PMC8636556/
S84	Andres et al. 2014 - structural basis of chitin oligosaccharide deacetylation (VcCDA) - Angew. Chem.	Journal (Wiley)	https://onlinelibrary.wiley.com/doi/10.1002/ange.201400220
S88	Broad-specificity CDA from Aspergillus nidulans fgsc A4 - ResearchGate	Journal	https://www.researchgate.net/publication/316860823
S89	ArCE4A / NodB+VcCDA stacking (Hamer et al. cited) - plos one	Journal (plos)	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0187544
S102	Chitinase: diversity, limitations & trends in engineering (incl. W167A/W275A, hypertransglycosylating S. proteamaculans endochitinase)	Review (ScienceDirect)	https://www.sciencedirect.com/org/science/article/pii/S1573493518002011
S103	Zakariassen et al. 2009 - Aromatic residues in ChiA affect processivity, activity & biomass conversion (W167A abolishes processivity)	Journal (J Biol Chem / PubMed)	https://pubmed.ncbi.nlm.nih.gov/19244232/
S105	Family 18 chitinase-oligosaccharide interaction: ChiA subsite preference & product sizes (E315L hexasaccharide, PDB 1NH6)	Journal (PMC)	https://pmc.ncbi.nlm.nih.gov/articles/PMC1223757/
S110	Horn et al. 2006 - Endo/exo mechanism & processivity of family-18 chitinases (ChiA/ChiB processive, ChiC non-processive gives longer oligomers) - PubMed	Journal (FEBS J)	https://pubmed.ncbi.nlm.nih.gov/16420473/
S111	Horn et al. 2006 - same, full text	Journal (Wiley)	https://febs.onlinelibrary.wiley.com/doi/full/10.1111/j.1742-4658.2005.05079.x
S112	Zakariassen et al. 2009 - ChiA aromatic-residue mutants; (GlcNAc)2 remains the dominant product - PubMed	Journal (J Biol Chem)	https://pubmed.ncbi.nlm.nih.gov/19244232/
S116	Payne et al. 2012 - Hallmarks of processivity in S. marcescens chitinases (aromatic +1/+2 subsites) - PubMed	Journal (J Biol Chem)	https://pubmed.ncbi.nlm.nih.gov/22952223/
S118	BRENDA EC 3.5.1.136 - diacetylchitobiose non-reducing-end deacetylase (Dac/TkCDA), T. kodakarensis, UniProt Q6F4N1	Database (BRENDA)	https://www.brenda-enzymes.org/enzyme.php?ecno=3.5.1.136&UniProtAcc=Q6F4N1&OrganismID=5246
S121	Chitin-to-L-lysine consortium - published ChiB->chitobiose(+some DP3)->non-reducing-end deacetylase (TkCDA) pipeline (prior art)	Journal (Appl Microbiol Biotechnol)	https://link.springer.com/article/10.1007/s00253-021-11112-5
S124	Tanaka et al. - Dac + exo-glucosaminidase chitinolytic pathway in T. kodakarensis KOD1 - PubMed	Journal (J Biol Chem)	https://pubmed.ncbi.nlm.nih.gov/11210511/
S136	BRENDA EC 3.2.1.14 (S. marcescens) - ChiC nonprocessive endochitinase, continuum of odd/even oligomers	Database (BRENDA)	https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.14&UniProtAcc=P11797&OrganismID=5690
S137	Suzuki et al. - Expression & characterization of Endochitinase C from S. marcescens BJL200 (endo, non-processive)	Journal (ResearchGate)	https://www.researchgate.net/publication/5528006
S138	Endochitinase C BJL200 - kinetics vs ChiA/ChiB and product range (GlcNAc2 main + GlcNAc3/4/5)	Journal (Biosci Biotechnol Biochem)	https://www.tandfonline.com/doi/abs/10.1271/bbb.70594
S141	Carbohydrate-binding modules of ChiB and ChiC promote the chitinolytic system of S. marcescens (CBM12 aids crystalline chitin)	Journal (ScienceDirect)	https://www.sciencedirect.com/science/article/abs/pii/S0141022922001375
S170	Chen/Zhao et al. - linker library (flexible GGGGS + rigid EAAAK); optimum often a combination; MD/fret	Journal (amb/Biochemistry)	https://link.springer.com/article/10.1007/s00253-015-6985-3
S172	iGEM BBa_K4990005 (2023) - flexible (GGGGS)n vs rigid (EAAAK)n linker design for bifunctional enzymes	Registry (parts.igem.org)	https://parts.igem.org/Part:BBa_K4990005

ID	Reference (short)	Type	URL
S173	Retracted chitinase-protease bifunctional fusion (Sm chitinase + Bc protease, G4S linker, ompA secretion) - use with caution	Journal (PLoS one; retracted)	https://pmc.ncbi.nlm.nih.gov/articles/PMC8975109/
S174	Fungal CDA (Puccinia graminis) expressed as MBP-fusion in E. coli periplasm -> soluble, defined paCOS	Journal (mBio)	https://pmc.ncbi.nlm.nih.gov/articles/PMC5086558/
S175	PpChi (Paenibacillus pabuli) - defined single-N-acetyl reducing-end COS; kcat 67.2/s; lobster shell; +AA11 LPMO 6x	Journal (J Agric Food Chem)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8041281/
S180	iGEM BBa_K1913000 (Wageningen 2016) - Sm ChiA; expression could not be detected	Registry	https://parts.igem.org/Part:BBa_K1913000
S181	iGEM BBa_K1201000 (2013) - Sm ChiA; active only with ChiB+ChiC	Registry	http://parts.igem.org/Part:BBa_K1201000:Design
S182	iGEM Uprize-I 2025 - four chitinase parts incl. Sm gei ChiA, expression verified	Team wiki	https://2025.igem.wiki/uprize-i/contribution
S183	iGEM TU Darmstadt 2017 (ChiTUcare) - NodB + COD orthogonal system for defined deacetylation	Team wiki	https://2017.igem.org/Team:TU_Darmstadt/project/chitin_deacetylase
S184	iGEM BBa_K2380041 - NodB (S. meliloti); inclusion bodies, refold, pH 9	Registry	https://parts.igem.org/Part:BBa_K2380041
S185	iGEM Stuttgart 2018 - BBa_K2848001 improved COD + NodC composite for chitosan	Team wiki	http://2018.igem.org/Team:Stuttgart/Composite_Part
S186	Tanaka et al. - Tk-Dac is an LmbE-family de-N-acetylase (not CE4); non-reducing GlcNAc of (GlcNAc)2-5	Journal (ResearchGate)	https://www.researchgate.net/publication/8568537
S187	BRENDA EC 3.5.1.136 - Dac deacetylates non-reducing residue vs COD (3.5.1.105) reducing residue of chitobiose	Database (BRENDA)	https://www.brenda-enzymes.org/enzyme.php?ecno=3.5.1.136&UniProtAcc=Q6F4N1&OrganismID=5246
S188	T. kodakarensis natural pathway: ChiA (TK1765) -> (GlcNAc)2 -> Dac (TK1764) - precedent for ChiA->Dac	Journal (aem/PMC)	https://pmc.ncbi.nlm.nih.gov/articles/PMC5514669/
S190	Tuveng et al. 2017 (PLoS one) - ArCE4A: broad specificity, higher activity on longer oligomers; bound (GlcNAc)2; internal sugars deacetylated first; ENA LT630322	Journal (plos)	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0187544
S191	Grifoll-Romero et al. review - ArCE4A order DP5>DP6~DP4>DP3>>DP2, Ni2+, multiple-chain, product pattern [Dn-1A]; AnCDA uses Co2+; CDAs low on crystalline chitin (LPMO enhances)	Journal (PMC)	https://pmc.ncbi.nlm.nih.gov/articles/PMC6415152/
S192	BRENDA EC 3.5.1.41 - ArCE4A: plain chitin poor substrate; Nitratireductor CDA3 active on crystalline chitin	Database (BRENDA)	https://www.brenda-enzymes.org/enzyme.php?ecno=3.5.1.41
S200	Chitosan Cu/Pb adsorption via free amino groups; Langmuir/Freundlich isotherms; pseudo-2nd-order kinetics	Journal (Carbohydr Polym)	https://www.sciencedirect.com/science/article/abs/pii/S0144861710000135
S201	Pt(IV) & Pd(II) adsorption on (amine-modified) chitosan using K2PtCl6 / PdCl2 - Pd/Pt salts as capture models	Journal (J Hazard Mater)	https://www.sciencedirect.com/science/article/abs/pii/S0304389410008022
S202	Cu(II) on chitosan/pva beads; Langmuir isotherm; EDTA desorbs bound metal; insoluble beads for handling	Journal (PubMed)	https://pubmed.ncbi.nlm.nih.gov/15225987/
S203	Shrimp-shell chitosan: degree of deacetylation by potentiometric titration & FTIR; Cu quantified by spectrophotometric titration + mip-oes	Journal (PMC)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8840202/
S204	Xps: metals bind chitosan via amino groups; amino-group availability is the key factor (crosslinking reduces it)	Journal (Colloids Surf A)	https://www.sciencedirect.com/science/article/pii/S0927775710006436