Supplementary material:

Structural characterization of the sporulation protein GerM from *Bacillus subtilis*

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Table S1

Mass spectrometry analysis of GerM recombinant constructs.

Construct	Theoretical MW (Da)	Experimental MW (Da)
GerMN1	15310.4	15310.9
$GerM_{26\text{-}366}$ (in solution)	37172.5	37173.4
$GerM_{26\text{-}366}$ (dissolved crystals)	37172.5	37173.1
$GerM_{26\text{-}366}$ (D202R-D203R)	37254.7	37255.4
$GerM_{26\text{-}366}$ (D219R)	37213.6	26706.1
$GerM_{26\text{-}366}$ (R246E)	37145.4	20340.5
$GerM_{26\text{-}366}$ (K252E)	37173.4	21932.0 and 21830.7
$GerM_{26\text{-}366}$ (K268E)	37173.4	23968.1 and 26066.5
$GerM_{26\text{-}366}$ (D275R)	37213.6	37214.4
$GerM_{26\text{-}366}$ (E278R)	37199.6	37200.0

Grey highlights indicate recombinant constructs that suffered from proteolytic degradation. MW, molecular weight

Table S2

Strains, plasmids and oligonucleotides used in this study.

Construct	Genotype/description/sequence	Source
***B. subtilis* strains**		
PY79	Prototrophic wild-type	Youngman, 1983
BCR287	*spoIIIAH::kan*	Rodrigues *et al.*, 2013
BCR1237	*gerM::erm*	Rodrigues *et al.*, 2016
BAT0044	*spoIIIAH::kan, gerM::erm*	This work
BAT0047	*gerM::erm, ycgO::P_{gerM}-gerM* (D275R) *(spec)*	This work
BAT0048	*spoIIIAH::kan, gerM::erm, ycgO::P_{gerM}-gerM* (D275R) *(spec)*	This work
BAT0075	*gerM::erm, ycgO::P_{gerM}-gerM* (D202R-D203R) *(spec)*	This work
BAT0076	*spoIIIAH::kan, gerM::erm, ycgO::P_{gerM}-gerM* (D202R-D203R) *(spec)*	This work
BAT0094	*gerM::erm, ycgO::P_{gerM}-gerM* (E278R) *(spec)*	This work
BAT0095	*gerM::erm, spoIIIAH::kan, ycgO::P_{gerM}-gerM* (E278R) *(spec)*	This work
Plasmids		
pCR280	*His6-SUMO-gerM*$_{26\text{-}366}$ (F26-F366, *B. subtilis*)	This work
pCR282	*His6-SUMO-gerMN1* (T76-E213, *B. subtilis*)	This work
pCR283	*His6-SUMO-gerMN2* (T223-F366, *B. subtilis*)	This work
pAT005	*His6-SUMO-gerM*$_{26\text{-}366}$ *(*K268E) (F26-F366, *B. subtilis*)	This work
pAT006	*His6-SUMO-gerM*$_{26\text{-}366}$ (D219R) (F26-F366, *B. subtilis*)	This work
pAT007	*His6-SUMO-gerM*$_{26\text{-}366}$ (E278R) (F26-F366, *B. subtilis*)	This work
pAT008	*His6-SUMO-gerM*$_{26\text{-}366}$ (R246E) (F26-F366, *B. subtilis*)	This work
pAT009	*His6-SUMO-gerM*$_{26\text{-}366}$ (K252E) (F26-F366, *B. subtilis*)	This work
pAT010	*His6-SUMO-gerM*$_{26\text{-}366}$ (D275R) (F26-F366, *B. subtilis*)	This work
pAT011	*His6-SUMO-gerM*$_{26\text{-}366}$ (D202R-D203R) (F26-F366, *B. subtilis*)	This work
pAT014	*ycgO::P_{gerM}-gerM (spec)*	This work
pAT019	*ycgO::P_{gerM}-gerM*(D275R) *(spec)*	This work
pAT023	*ycgO::P_{gerM}-gerM*(D202R-D203R) *(spec)*	This work
pAT026	*ycgO::P_{gerM}-gerM*(E278R) *(spec)*	This work
Oligonucleotides		
oAT012	ttggctaaagggccgagcgaggtgagcgggctgttaaca	This work
oAT013	tgttaacagcccgctcacctcgctcggccctttagccaa	This work
oAT014	gtgagcgggctgttaacacgcttcagtgaagatgtaaag	This work
oAT015	ctttacatcttcactgaagcgtgttaacagcccgctcac	This work
oAT016	gaaacagccggtgtaaatcgtctcactgctacccatccg	This work
oAT017	cggatgggtagcagtgagacgatttacaccggctgtttc	This work
oAT018	tacgtgccggtgaccaaagaaatcgacaattcggaaaaa	This work
oAT019	tttttccgaattgtcgatttctttggtcaccggcacgta	This work
oAT020	ggagggacgccgatctcacgtcgtttaagccgcaaagacggc	This work
oAT021	gccgtctttgcggcttaaacgacgtgagatcggcgtccctcc	This work
oAT022	cgaatcgacaattcggaagaagacgacatcacagcagcc	This work
oAT023	ggctgctgtgatgtcgtcttcttccgaattgtcgattcg	This work
oAT024	ctgttaacagacttcagtagagatgtaaagctggtcagc	This work
oAT025	gctgaccagctttacatctctactgaagtctgttaacag	This work
oAT058	cgcaagcttacagcccgggaagtcagcacaattc	This work
oAT059	cgcggatcccgattgtatgtacataaacca	This work
oCR607	gctcacagagaacagattggtggttttcaatctgataaggcagccgag	This work
oCR608	acggagctctgctcttctaccttaaaaactacccgtattcacttg	This work
oCR610	gctcacagagaacagattggtggtactgttatgagggagctttatctc	This work
oCR611	acggagctctgctcttctaccttattccaaattaatgccgtctttgcg	This work
oCR612	gctcacagagaacagattggtggtacccatccgctgacagtctacta	This work

Plasmid Construction

pCR280 [*His6-SUMO-gerM*$_{26\text{-}366}$ (residues F26 to F366 of GerM from *B. subtilis*)] was generated by isothermal assembly of a PCR product containing the relevant DNA segment of *gerM* (oligonucleotide primers oCR607 and oCR608 on PY79 genomic DNA) into pTB146 [*His6-SUMO*] cut with *Sap*I. pTB146 is a protein expression vector provided by T. Bernhardt, Harvard Medical School, Boston.

pCR282 [*His6-SUMO-gerMN1* (residues T76 to E213 of GerM from *B. subtilis*)] was generated by isothermal assembly of a PCR product containing the relevant DNA segment of *gerM* (oligonucleotide primers oCR610 and oCR611 on PY79 genomic DNA) into pTB146 [*His6-SUMO*] cut with *Sap*I.

pCR283 [*His6-SUMO-gerMN2* (residues T223 to F366 of GerM from *B. subtilis*)] was generated by isothermal assembly of a PCR product containing the relevant DNA segment of *gerM* (oligonucleotide primers oCR612 and oCR608 on PY79 genomic DNA) into pTB146 [*His6-SUMO*] cut with *Sap*I.

pAT005 [*His6-SUMO-gerM*$_{26\text{-}366}$(K268E) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT012 and oAT013 on pCR280.

pAT006 [*His6-SUMO-gerM*$_{26\text{-}366}$(D219R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT016 and oAT017 on pCR280.

pAT007 [*His6-SUMO*-gerM$_{26\text{-}366}$(E278R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT024 and oAT025 on pCR280.

pAT008 [*His6-SUMO-gerM*$_{26\text{-}366}$(R246E) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT018 and oAT019 on pCR280.

pAT009 [*His6-SUMO-gerM*$_{26\text{-}366}$(K252E) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT022 and oAT023 on pCR280.

pAT010 [*His6-SUMO-gerM*$_{26\text{-}366}$(D275R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT014 and oAT015 on pCR280.

pAT011 [*His6-SUMO-gerM*$_{26\text{-}366}$(D202R-D203R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT020 and oAT021 on pCR280.

pAT014 [*ycgO*::P_{gerM}-*gerM* (spec)] was generated by a two-way ligation of a EcoR1-BamHI of a PCR product (oligonucleotide primers oAT0058 and oAT0059 on PY79 genomic DNA) containing the relevant DNA segment of GerM into pKM083 cut with *Eco*RI-*Bam*HI. pKM083 is an ectopic integration vector for recombination into the *ycgO* locus (David Z. Rudner and K. Marquis, Harvard Medical School, Boston).

pAT019 [*ycgO*::P_{gerM}-*gerM*(D275R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT014 and oAT015 on pAT014.

pAT023 [*ycgO*::P_{gerM}- *gerM*(D202R-D203R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT020 and oAT021 on pAT014.

pAT026 [*ycgO*::P_{gerM} *gerM*(E278R) (F26-F366, *B. subtilis*)] was generated by site-directed mutagenesis using oligonucleotide primers oAT024 and oAT025 on pAT014.

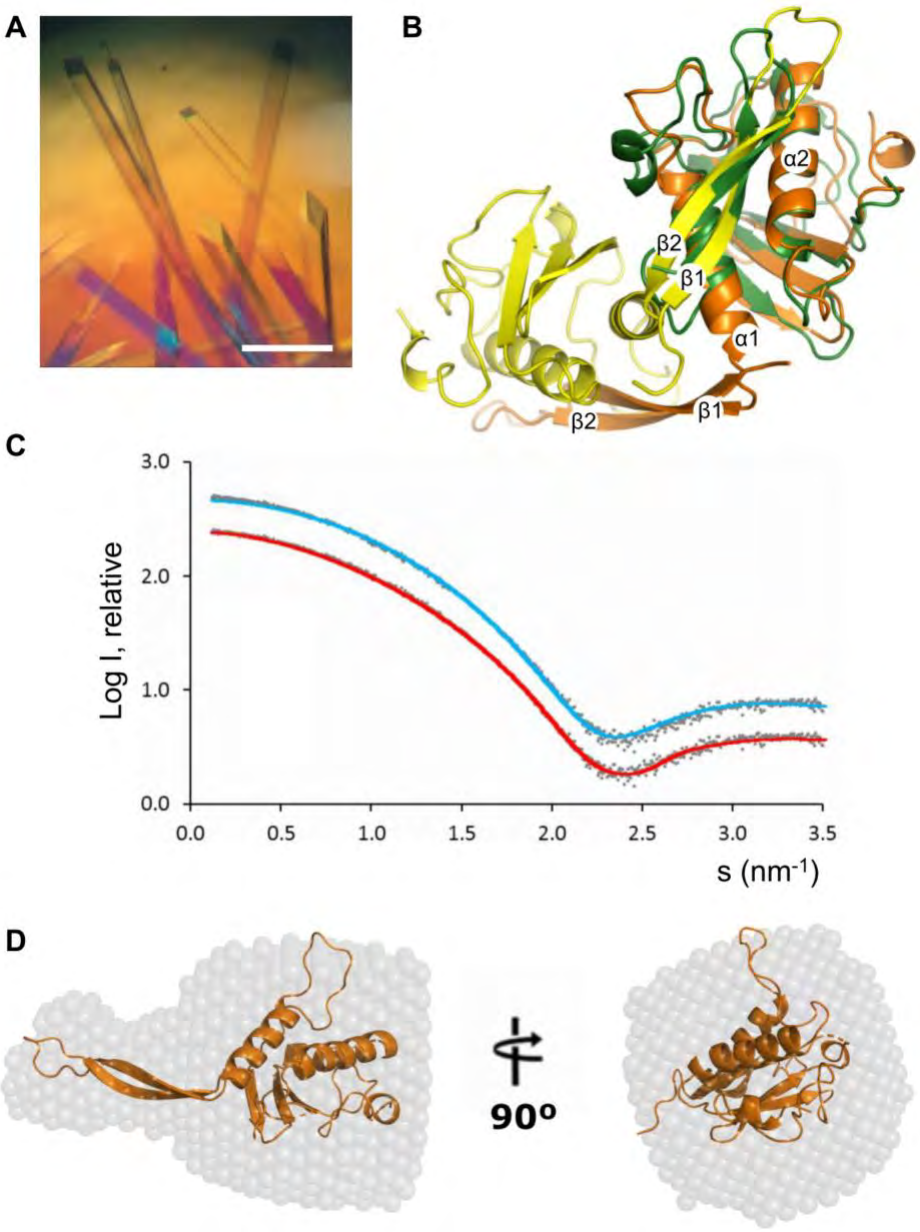

Figure S1. Structural characterization of GerMN1. A. Needle-shaped crystals of GerMN1 obtained in 200 mM Na acetate, 21% (w/vol) PEG 3,350. Scale bar, 100 µm. **B.** Overlay of the GerMN1 domain (in green) from *B. subtilis* GerM with a dimer present in the asymmetric unit of the $GerMN_{Cp}$ crystal (PDB entry 5J7R). Note that the core of GerMN1 superposes with the globular core of one $GerMN_{Cp}$ molecule (in orange), while the β1β2 sheet of GerMN1 superposes with the equivalent β-sheet of the other $GerMN_{Cp}$ molecule (in yellow). **C.** Scattering data and model fits for GerMN in solution. The graph displays the GerMN experimental scattering data (grey dots) and the fitting of the scattering curves computed from the *ab initio* GerMN1 DAMMIF model (red curve) or from the GerMN1 crystal structure (blue curve). **D.** $GerMN_{Cp}$ crystal structure (orange cartoon) fitted into the DAMMIF generated envelope of GerMN1 in solution (grey spheres).

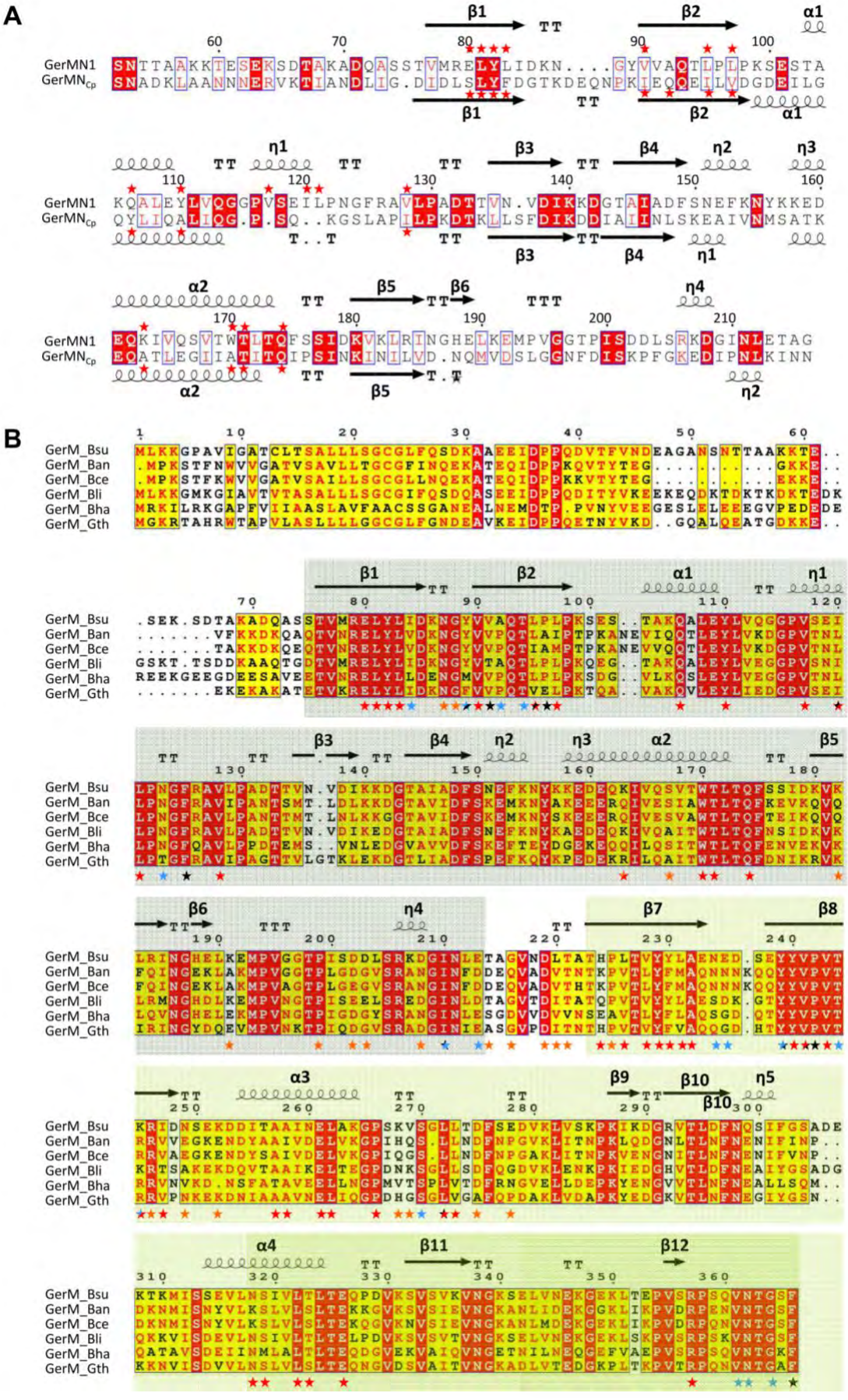
A
GerMN1
GerMN$_{Cp}$
B
GerM_Bsu
GerM_Ban
GerM_Bce
GerM_Bli
GerM_Bha
GerM_Gth

Figure S2. Position and conservation of residues located at intramolecular and intermolecular interfaces of GerM. A. Alignment of the sequences of GerMN1 from *B. subtilis* GerM and $GerMN_{Cp}$ from *C. perfringens.* Conserved residues are in red boxes; similar residues are shown by red letters boxed in blue. The secondary structures of GerMN1 and $GerMN_{Cp}$ are indicated above and below the sequence alignment, respectively. Residues at the interface between the β1β2 sheet and the core of the GerMN domain are indicated by red stars. **B.** Alignment of the GerM sequences from *B. subtilis* ($GerM_{Bsu}$), *B. anthracis* ($GerM_{Ban}$), *B. cereus* ($GerM_{Bce}$), *B. licheniformis* ($GerM_{Bli}$), *B. halodurans* ($GerM_{Bha}$) and *G. thermodenitrificans* ($GerM_{Gth}$). Conserved residues are in red boxes; similar residues are shown by red letters boxed in yellow. The secondary structures of $GerM_{Bsu}$ are indicated above the sequence alignment. Residues at the GerMN1-GerMN2 interface are indicated by black stars. Residues at the homodimerization interface are indicated by blue stars. Residues at the interface between the β1β2 sheet and the core of the GerMN domain are indicated by red stars. Arrows indicate β-strands; α, α-helices; η, 3_{10}-helices.

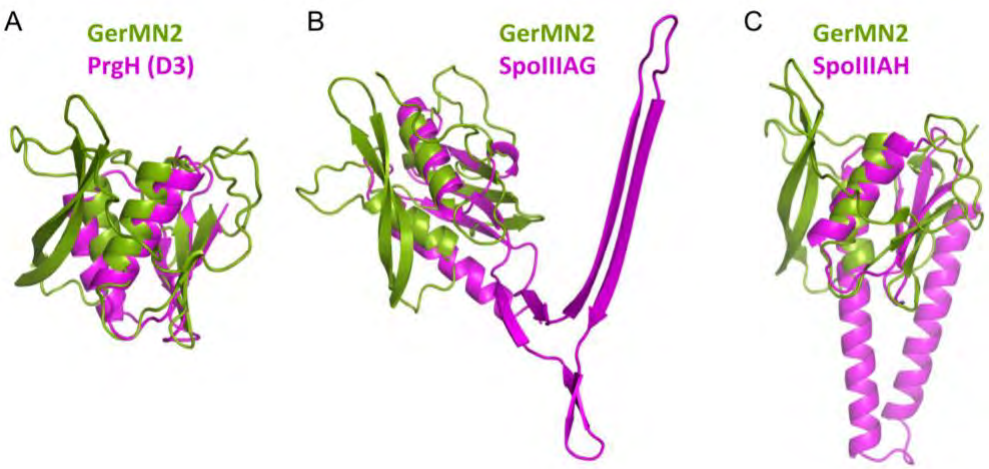

Figure S3. Comparison of GerMN2 with RBM domains. A-C. Ribbon representations of GerMN2 (in light green) superimposed onto the second periplasmic RBM (D3) of PrgH from *S. typhimurium* (**A**, PDB code 4G1I, in magenta), and onto SpoIIIAG (**B**, PDB code 5WC3, in magenta) and SpoIIIAH (**C**, PDB code 3UZ0, in magenta) from *B. subtilis*.

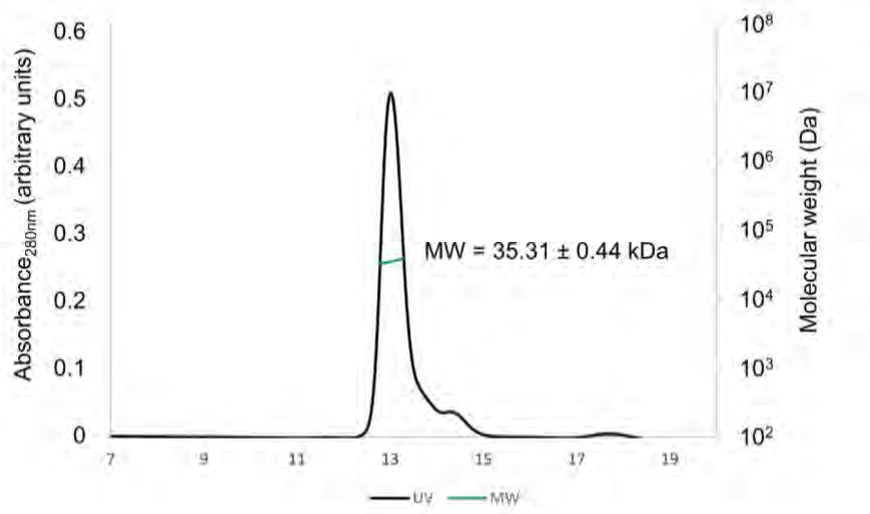

Figure S4. SEC-MALLS analysis of GerM$_{26\text{-}366}$ in solution. Chromatograms are displayed with the absorbance at 280 nm as a black line and arbitrary units displayed on the left axis, and molecular weight estimation as a green line with values (in Da) displayed on the right axis. The estimated average molecular weight (MW) is detailed on the graph.