
Phase transition of GvpU regulates gas vesicle clustering in bacteria

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Supplementary Tables 1.**Detailed amino acid sequence of GvpA_{Ana} and GvpA2_{Mega} fragment.**

Name	Sequence Range	Sequence
A _{Ana} Nt	M1-S10	MAVEKTNSSSS
A2 _{Mega} Nt	M1-S10	MSIQKSTNSS
A _{Ana} α1	A13-G23	AEVIDRILDKG
A2 _{Mega} α1	A13-G23	AEVIDRILDKG
A _{Ana} β1	V25-L34	VIDAWVRVSL
A2 _{Mega} β1	V25-V34	VIDAFARVSV
A _{Ana} β2	E38-S50	ELLAIEARIVIAS
A2 _{Mega} β2	E38-S50	EILTIEARVVIAS
A _{Ana} α2	V51-V60	VETYLKYAEAV
A2 _{Mega} α2	V51-V60	VDTWLRYAEAV
A _{Ana} Ct	T64-A71	TQSAAVPA
A2 _{Mega} Ct	L64-I88	LRDDVEENGLPERSNSSEGQPRFSI

Supplementary Methods

1. Sequences of oligonucleotides

Oligonucleotides_Name	Sequence	Comment
ZL202_gvp_fwd	aatgggatcaTAAGCGAAAGG AGAAATG	Gibson assembly to make $\Delta gvpU$ from pNL29 WT operon.
ZL202_gvp_rev	ggctttgtaTTAGGCTGTTGTA TCGTC	
ZL202_vector_fwd	aacagcctaaTAACAAAGCCC GAAAGGAAG	
ZL202_vector_rev	cttcgcttaTGATCCCATTGTTT TTAAATCTG	
Delta_GvpR_fwd	ACGATAAGATGGCAGGAG	KLD to make $\Delta gvpR$ from pNL29 WT operon.
Delta_GvpR_rev	ATAGTTAGATCTAAAAGCTG GAACC	
Delta_GvpT_rev	GACGTAAAGGAGGAAAGA AAG	KLD to make $\Delta gvpT$ from pNL29 WT operon.
Delta_GvpT_fwd	TTTTTTAAGTAGCGTACAGT TTTAAAC	
GvpU_fwd	GACGTAAAGGAGGAAAGA AAG	KLD to make <i>gvpU</i> from pNL29 WT operon.
GvpU_rev	TTCGCTTATGATCCCATTG	
GvpU::His_fwd	caccaccacTAAAAACGGCG GGGTGA	KLD to make <i>gvpU::His₆-tag</i> from <i>gvpU</i> construct.
Gvp::His_rev	atgatgatgTGAAGTCTTTTTA CTCGTTGATTTTG	
GL094_BB_A2C_fwd	tctaactattTTCCTTTTTTGAG GGTGG	Gibson assembly to make ARG1 gene clusters in pST39 vector as A2C.
GL094_BB_A2C_rev	ggaaaaccctATTGTTTTTAAA TCTGTAGATGATTTAATATC	
GL100_A2C_fwd	taaaaacaatAGGGTTTTCCC AGTCGAG	
GL100_A2C_rev	aaaaaaggaaAATAGTTAGAT CTTTATTAACCAAAGATACT C	
A2_fwd	TAAAGATCTAACTATTTTCC TTTTTTG	KLD to make AWT from ARG1 gene clusters in pST39.
A2_rev	GAAGTTCTCCAAAAAATAG TAAATTAG	
A2DeltaU_fwd	AAAACGGCGGGGTGATTG	KLD to make AWT $\Delta gvpU$ from AWT and B2C $\Delta gvpU$ from B2C.
A2DeltaU_rev	TTACGTCTTAGGCTGTTGT ATC	
ZL215_vector_fwd	taaaagcgctCATCATCATCAC CACCAC	Gibson assembly to make <i>gvpU::sfGFP</i> from <i>gvpU</i> and <i>sfGFP</i> gene containing plasmid.
ZL215_vector_rev	ttgaaccgccTGAAGTCTTTTT ACTCGTTG	

ZL215_fragment_fwd	aaagagttcaGGCGGTTCAAA AGGTGAAG	Gibson assembly to make pNL29 WT with <i>gvpU::mCherry</i> from pNL29 WT operon and <i>mCherry</i> gene containing plasmid.
ZL215_fragment_rev	gatgatgatgAGCGCTTTTATAT AATTCATCCATAC	
ZL262_vector_fwd	gctgtacaagTAAAAAACGGC GGGGTGATTG	
ZL262_vector_rev	ctgtactcatGTCTTTCTTTCCT CCTTTACGTC	
ZL262_fragment_fwd	aaagaaagacATGAGTACAGG CCCTTCTTTTC	
ZL262_fragment_rev	ccggtttttaCTTGTACAGCTCG TCCATG	
GL094_BC_fwd	atctaactatggaaccttccttttgag	Gibson assembly to make BC from pNL29 WT operon and ARG1 gene clusters.
GL094_BC_rev	taatcctgatataccacttcatattaaat actaaaac	
GvpC_100_fwd	gaagtggatatacaggattaattaatcct gatattg	
GvpC_100_rev	ggaaggttccatagttagatctttattaa ccaaagatac	
ZL088_B2C_fwd	CCTGATATTGGTTCTGCTT TTTTTAATTATC	Gibson assembly to make B2C from BC.
ZL088_B2C_rev	tttaaactcgATTAATTAATCCTG ATATACCACTTCATATTAAAT AC	
GvpB_94_fwd	ggattaattaatCAGATTTAAAAA CAATGGGATC	
GvpB_94_rev	GCAGAACCAATATCAGGAT TAATTAA	
pST_Backbone_fwd	attatataaaTAAAAAACGGCG GGGTGATTG	Gibson assembly to make <i>sfGFP (Neg.)</i> from pNL29 WT operon and <i>sfGFP</i> containing plasmid (Addgene: #107420).
pST_Backbone_rev	caccttttgaCATGTCTTTCTTT CCTCCTTTACG	
sfGFP_fwd	gaaagacatgTCAAAAGGTGA AGAATTATTTAC	
sfGFP_rev	gccgtttttaTTTATATAATTCAT CCATACCATGTG	
ZL130_fwd	gcagtacctgctTAAAAAACGGC GGGGTGATTGCC	KLD to make <i>sfGFP::A_{Ct}</i> from <i>sfGFP</i> .
ZL130_rev	tgctgattgggtGCTGCCACCTC CGCCTCC	
ZL131_fwd	cgcatataagccgacgaagTAAAA AACGGCGGGGTGATTGCC	KLD to make <i>sfGFP::SpyTag (Pos.)</i> from <i>sfGFP</i> .
ZL131_rev	tctaccattacaatatgtgcGCTGC CACCTCCGCCTCC	
ZL129_All_fwd	tttagtattTAAAAAACGGCGG GGTGATTGCC	Gibson assembly to make <i>sfGFP::B_{Ct}</i> from pNL29 WT operon and <i>sfGFP</i> .
ZL129_All_rev	cgtcacgaagGCTGCCACCTC CGCCTCC	

GvpB_peptide_fwd	aggtggcagcCTTCGTGACGA CGTAGAAG	
GvpB_peptide_rev	ccgtttttaAATACTAAAACGCG GCTG	
ZL144_fwd	gcagaagcagtagggcttCTTCGT GACGACGTAGAAGAAAAC GGTC	KLD to make <i>sfGFP::B_{α2-Ct}</i> from <i>sfGFP::B_{Ct}</i> .
ZL144_rev	atagcgtaaccatgtatcGCTGCC ACCTCCGCCTCC	
ZL145_129_fwd	agataaaggaTAAAAACGGC GGGGTGATTGCC	Gibson assembly to make <i>sfGFP::B_{Nt-α1}</i> from pNL29 WT operon and <i>sfGFP</i> .
ZL145_129_rev	gaatagacatGCTGCCACCTC CGCCTCC	
GvpB_first_Helix_fwd	aggtggcagcATGTCTATTCAA AAAAGTACTAATAG	
GvpB_first_Helix_rev	ccgtttttaTCCTTTATCTAAAA TACGGTC	
ZL146_129_fwd	tgccagtgtTAAAAACGGCG GGGTGATTGCC	Gibson assembly to make <i>sfGFP::B_{Nt-β2}</i> from pNL29 WT operon and <i>sfGFP</i> .
ZL146_129_rev	gaatagacatGCTGCCACCTC CGCCTCC	
GvpB_first_Helix+2bs_fwd	aggtggcagcATGTCTATTCAA AAAAGTACTAATAG	
GvpB_first_Helix+2bs_rev	ccgtttttaAACACTGGCAATA ACCAC	
MBP_GvpB_AA066_fwd	aggtggcagcAAAATCGAAGA AGGTAAACTG	Gibson assembly to make <i>sfGFP::MBP::gvpB</i> from <i>sfGFP</i> and <i>MBP::gvpB</i> gene containing plasmid.
MBP_GvpB_AA066_rev	ccgtttttaAATACTAAAACGCG GCTG	
sfGFP_ZL129_fwd	tttagtattTAAAAACGGCGG GGTGATTGCC	
sfGFP_ZL129_rev	cttcgatttGCTGCCACCTCCG CCTCC	
ZL273_Vector_fwd	gagttcataaAAAACGGCGGG GTGATTG	Gibson assembly to make pNL29 WT with <i>gvpU</i> <i>mutation (F->S; E->S, G; F,</i> <i>E-> S, G)</i> from pNL29 WT operon and gBlocks of GvpU with those mutations.
ZL273_Vector_rev	ctgtactcatGTCTTTCTTTCCT CCTTTACGTC	
ZL273_Fragment_fwd	aaagaaagacATGAGTACAGG CCCTTCTTTTTC	
ZL273_Fragment_rev	aaagaaagacATGAGTACAGG CCCTTCTTTTTC	
GL094_WO-GvpB_fwd	cccgcctgattatgaagtggatggaa cc	Gibson assembly to make <i>B_{Nt-α2}A_{Ct}</i> from pNL29 WT operon and <i>B_{Nt-α2}A_{Ct}</i> gBlock.
GL094_WO-GvpB_rev	gaatcgacatatctatttctctttcg	
ZL112_fwd	gaaatgagatatgtcgattcagaagtc aaccaattcttc	
ZL112_rev	ccacttcataatcaggcgggtacggca g	

ZL106_Entire_fwd	ctgattttttatctagatatttgaaatca acg	Gibson assembly to make $B_{Nt-\alpha 2}A_{Ct}2C$ from BC and $B_{Nt-\alpha 2}A_{Ct}$ gBlock.
ZL106_Entire_rev	cattgtttttaaataaaaaaatcag gcgg	
ZL106_GvpB-fragment_rev	tttttaattttaaaaacaatgggatcat aag	
ZL106_GvpB-fragment_fwd	tatctagataaaaaaatcaggcgggt ac	
ZL090_NoGvpA2_fwd	tttagtatttaaTTTAATTAAGCTC CATAATATCAGG	Gibson assembly to make $A_{Nt-\alpha 2}B_{Ct}2C$ from A2C operon and $A_{Nt-\alpha 2}B_{Ct}2C$ gBlock.
ZL090_NoGvpA2_rev	caacggccatTTTTTTAATTCCT TGTTATTTGTTG	
ZL107_fwd	aattaaaaaaATGGCCGTTGA GAAGACTAATAG	
ZL107_rev	cttaattaaaTTAAATACTAAAA CGTGGTTGACC	
ZL119_fwd	TTTTCCTTTTTTGAGGGTG	KLD to make $A_{Nt-\alpha 2}B_{Ct}$ from $A_{Nt-\alpha 2}B_{Ct}2C$.
ZL119_rev	GCTTAATTGAATCAGATAGA G	
ZL157_fwd	aaaagtactaatTCGAGTTCCC TGCGGAG	KLD to make $B_{Nt}A_{\alpha 1-\alpha 2}B_{Ct}$ from $A_{Nt-\alpha 2}B_{Ct}$.
ZL157_rev	ttgaatagacatTTTTTTAATTCC TTGTTATTTGTTGTCGGAA AC	
94_158_fwd	tattgcctctGTTGATACATGGT TACGC	Gibson assembly to make $A_{Nt-\beta 2}B_{\alpha 2-Ct}$ from pNL29 WT operon and $A_{Nt-\alpha 2}B_{Ct}$.
94_158_rev	caacggccatATCTCATTTCTC CTTTCG	
119_158_fwd	gaaatgagatATGGCCGTTGA GAAGACTAATAG	
119_158_rev	atgtatcaacAGAGGCAATAAC AATACGAG	
ZL159_fwd	aagactaatagtTCTTCGTCACT GGCTGAG	KLD to make $A_{Nt}B_{\alpha 1-\alpha 2}A_{Ct}$ from $B_{Nt-\alpha 2}A_{Ct}$.
ZL159_rev	ctcaacggccatATCTCATTTCT CCTTTCGC	
105_160_fwd	tattgccagtGTTGATACTTGGT TACGCTATGCCGAG	Gibson assembly, followed by KLD to make $B_{Nt-\beta 2}A_{\alpha 2-Ct}$ from pNL29 WT operon and $B_{Nt-\alpha 2}A_{Ct}$.
105_160_rev	ccacttcataTCAGGCGGGTAC GGCAGC	
94_160_fwd	acccgcctgaTATGAAGTGGTA TGGAACC	
94_160_rev	aagtatcaacACTGGCAATAAC CACTCG	
ZL161_rev	cttgaagTATGCCGAGGCGGT TGTT	
ZL161_fwd	taggtttcAACACTGGCAATAA CCTACTCG	

2. Reagent or kit catalog number

Reagent or kit	Company	Catalog #
NEBuilder® HiFi DNA Assembly Master Mix	New England Biolabs (NEB), Ipswich, MA	E2621X
KLD Enzyme Mix	New England Biolabs (NEB), Ipswich, MA	M0554S
Q5® Hot Start High-Fidelity 2X Master Mix	New England Biolabs (NEB), Ipswich, MA	M0494X
BL21 Star™ (DE3)pLysS One Shot™ Chemically Competent E. coli	Thermo Fisher Scientific, Waltham, MA	C602003
LB Medium, Miller	Thermo Fisher Scientific, Waltham, MA	BP9723-2
Carbenicillin (Disodium), USP Grade	Gold Biotechnology, Olivette, MO	C-103-5
Chloramphenicol	MilliporeSigma, Burlington, MA	C0378-25G
D-(+)-Glucose	MilliporeSigma, Burlington, MA	G7021
IPTG Solutions	Teknova, Hollister, CA	I3502
Genlantis™ SoluLyse™ Bacterial Protein Extraction Reagent	Genlantis, San Diego, CA	L200500
Lysozyme	MilliporeSigma, Burlington, MA	L6876
Deoxyribonuclease I RNase-free solution from bovine pancreas	MilliporeSigma, Burlington, MA	D7291-2MG
20X PBS Solution. 1000mL, Sterile	Teknova, Hollister, CA	P0191
Benzonase® Nuclease, ultrapure	MilliporeSigma, Burlington, MA	E8263-25KU
DL-Dithiothreitol	MilliporeSigma, Burlington, MA	43815
Ni-NTA Agarose (500 ml)	Qiagen USA, Germantown, MD	30250
Pierce™ Disposable Columns, 10 mL	Thermo Fisher Scientific, Waltham, MA	29924
4–15% Mini-PROTEAN® TGX™ Precast Protein Gels, 10-well, 50 µl	Bio-Rad Laboratories, Hercules, CA	4561084
4–15% Mini-PROTEAN® TGX™ Precast Protein Gels, 15-well, 15 µl	Bio-Rad Laboratories, Hercules, CA	4561086
Coomassie Brilliant Blue G-250	Bio-Rad Laboratories, Hercules, CA	1610406
Formvar, 200 mesh, copper	Ted Pella, Redding, CA	01700-F

Karnovsky'S Fixative	Electron Microscopy Sciences, Hatfield, PA	15720
2% Uranyl Acetate Solution	Electron Microscopy Sciences, Hatfield, PA	22400-2
Whatman® qualitative filter paper, Grade 1	MilliporeSigma, Burlington, MA	WHA1001325
Cuvettes, macro and semi-micro, PS, CERTIFIED LIFE SCIENCE QUALITY	BRANDTECH® Scientific Inc, Essex, CT	759075D
6x-His Tag Monoclonal Antibody (HIS.H8), HRP	Thermo Fisher Scientific, Waltham, MA	MA1-21315-HRP
Pierce™ ECL Western Blotting Substrate	Thermo Fisher Scientific, Waltham, MA	32209
1,6-Hexanediol, 97%,	Thermo Fisher Scientific, Waltham, MA	A12439.0E
ATP Solution (100 mM)	Thermo Fisher Scientific, Waltham, MA	R0441
Pierce™ His Protein Interaction Pull-Down Kit	Thermo Fisher Scientific, Waltham, MA	21277
Corning® 96-well Half Area Black Flat Bottom Polystyrene NBS Microplate	Corning Inc, Corning, NY	3686
Hellma Compact-cuvette 176.700-QS, 1 mm layer thickness	Hellma GmbH & Co. KG, Müllheim, Germany	HELLMA_Z0228
Amicon® Ultra Centrifugal Filter, 10 kDa MWCO	MilliporeSigma, Burlington, MA	UFC901024
35 mm Dish No. 1.5 Coverslip 14 mm Glass Diameter Uncoated	MatTek Corporation, Ashland, MA	P35G-1.5-14-C
HBSS, calcium, magnesium, no phenol red	Thermo Fisher Scientific, Waltham, MA	14025092