Supplementary information for

Construction of recombinant Pdu metabolosome shells for small molecule production in *C. glutamicum*

Isabel Huber[†], David J. Palmer[‡], Kira N. Ludwig[†], Ian R. Brown[‡], Martin J. Warren[‡] and Julia Frunzke[†*]

[†]Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, Jülich, Germany

[‡]School of Biosciences, University of Kent, Giles Lane, Canterbury, Kent CT2 7NJ, U.K.

*Address correspondence to j.frunzke@fz-juelich.de

Construction of plasmids

For the construction of the Pdu production and integration plasmids, the plasmids pJP063 containing *pduABJKNUT* [1] and pED460 containing *pduA-X* [2] were used as initial PCR templates. All constructs derived from those plasmids are listed and construction procedures were described in Table S2. The DNA template 'Protein_scaffolds$_{opt}$' was synthesized (Sequence S1) and used as described in Table S4. The construction of different fluorescence reporter production plasmids is described in Table S5. Genomic template DNA from *Zymomonas mobilis subsp. mobilis* ATCC 29191 was kindly provided by Stephanie Bringer-Meyer and used for the amplification of the enzymes alcohol dehydrogenase B (AdhB; GenBank: AFN57379.1) and the pyruvate decarboxylase (Pdc; GenBank: AFN57569.1) All derived plasmids are listed in Table S6.

Compartment purification from MB001(DE3) *pduABJknt*

The compartments produced by MB001(DE3) *pduABJknt* were attempted to be purified using a protocol based on the compartment insolubility in YPER plus reagent and salt precipitation [3]. In the final supernatant fraction, three of six compartment shell proteins were detected by MALDI-MS (Fig. S3).

Material and Methods - Metabolosome purification

For compartment purification from *C. glutamicum* MB001(DE3) *PduABJknt*, 200 mL CGXII medium supplemented with 2% glucose and 50 µM IPTG were inoculated with precultures to an OD_{600} of 0.5 and cultivated for 16 h at 25 °C. Before cell lysis, the cells were harvested and washed with lysozyme buffer (50 mM Tris-HCl, pH 8, 5 mM EDTA, 0.6 M sucrose, 0.2% 1,2-propanediol, w/o lysozyme). The cell pellet was resuspended in 100 mL lysozyme buffer (containing 2 mg ml^{-1} lysozyme) and incubated for 1 h at room temperature. Afterwards, the cells were washed with lysozyme buffer and re-suspended in 50 mL Y-PER™ Plus Dialyzable Yeast Protein Extraction Reagent (Thermo Fischer Scientific, Waltham, USA) supplemented with EDTA-free Protease Inhibitor Cocktail tablets (Roche Diagnostics,

Mannheim, Germany) and Benzonase® nuclease and incubated at room temperature for 1 h. The cell suspension was sonicated for 10 minutes with 1 minute sonication (Amplitude: 80%; Output: 8; Branson Sonifier 250 G; Heinemann Ultraschall- und Labortechnik, Schwäbisch Gmünd, Germany) and 1 minute cooling intervals. Cell debris and intact cells were separated from cell lysate by centrifugation at 4000 *g* for 15 min at 4 °C. Starting from the cell lysate, the protocol from Lawrence *et al.* was followed [3]. In contrast to the original protocol, the NaCl concentration was raised to 160 mM NaCl (instead of 80 mM) to precipitate the compartment shells.

The different protein fractions were separated using 4-20% Mini-PROTEAN® TGX™ Precast Protein Gels (Biorad, Hercules, USA) in the Mini-PROTEAN Tetra Cell System (Bio-Rad). Precision Plus Protein™ Dual Color Standard (Bio-Rad, Hercules, USA) was used as protein standard and gels were stained with Rapid Stain™ (G-Biosciences, St. Louis, USA).

Figures and Tables

Sequence S1: Synthesized 'Protein_scaffolds$_{opt}$' sequences.

> GBD_{lig}
CTGGTGGGCGCACTGATGCACGTGATGCAGAAGCGCTCCCGCGCAATCCACTCCTCCGATGAAGGCGAAGA
TCAGGCAGGCGATGAAGATGAAGAT
> $SH3_{dom}$
GCAGAGTATGTGCGTGCCCTCTTTGACTTTAATGGTAATGATGAAGAAGATCTTCCCTTTAAGAAAGGAGACA
TCCTGCGCATCCGCGATAAGCCTGAAGAGCAGTGGTGGAATGCAGAGGACAGCGAAGGAAAGCGCGGTAT
GATTCCTGTCCCTTACGTGGAGAAGTATCGC
> PDZ_{dom}
CTCCAGCGTCGCCGCGTGACGGTGCGCAAGGCCGACGCCGGCGGTCTGGGCATCAGCATCAAGGGTGGC
CGTGAAAACAAGATGCCTATTCTCATTTCCAAGATCTTCAAGGGACTGGCAGCAGACCAGACGGAGGCCCTT
TTTGTTGGTGATGCCATCCTGTCTGTGAATGGTGAAGATTTGTCCTCTGCCACCCACGATGAAGCGGTACAG
GCCCTCAAGAAGACCGGCAAGGAGGTTGTGTTGGAGGTTAAGTACATGAAGGAGGTCTCACCCTATTTCAAG
> GBD_{dom}
ACCAAGGCAGATATTGGAACTCCATCCAATTTCCAGCACATTGGACATGTTGGTTGGGATCCAAATACCGGTT
TTGATCTAAATAATTTGGATCCAGAATTGAAGAATCTTTTTGATATGTGTGGTATCTCTGAGGCCCAGCTTAAA
GACCGCGAAACTTCAAAAGTTATTTATGACTTTATTGAAAAAACTGGAGGTGTAGAAGCTGTTAAAAATGAACT
CCGTCGCCAAGCACCA
> $C17_{P.m.}$ with $(GGGS)_2GG$ linker
GGCGGTGGCTCCGGCGGCGGTTCCGGCGGTACCGAAGAAAACGTGGAACGCATCATCAAGGAAGTGCTGG
GCCGCCTGGGCAAG

Table S1: Strains and plasmid used within this work. **pdu* genes marked in lower case have an exchanged start codon from ATG to GTG/TTG.

Strain or plasmid	Relevant characteristics	Source or reference
E. coli		
DH5α	F^- *endA1* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *recA1 relA1 hsdR17*(r_K^- m_K^+) *deoR supE44 thi-1 gyrA96 phoA* λ^-; strain used for general cloning procedures	[4]
C. glutamicum		
MB001	Type strain ATCC 13032 with deletion of prophages CGP1 (cg1507-cg1524), CGP2 (cg1746-cg1752), and CGP3 (cg1890-cg2071)	[5]
MB001(DE3)	MB001 derivative with chromosomally encoded T7 gene 1 (cg1122-P_{lacI}-*lacI* P_{lacUV5} –*lacZ*α-T7 gene *1*-cg1121)	[6]
MB001(DE3)::P_{T7}*pduABJKNUT*	MB001(DE3) derivative with *pduABJKNUT* from *C. freundii* under control of P_{T7} in the CGP1 region	This work
MB001(DE3)::P_{T7}*pduA*	MB001(DE3) derivative with *pduA* from *C. freundii* under control of P_{T7} in the CGP1 region	This work
MB001(DE3)::P_{T7}*pduABJknt**	MB001(DE3) derivative with *pduABJknt* from *C. freundii* under control of P_{T7} in the CGP1 region	This work
Plasmids		
pAN6	Kan^R; *C. glutamicum*/*E. coli* shuttle plasmid for regulated gene expression using P_{tac} (P_{tac} *lacI*^{q} pBL1 *ori*V_{Cg} pUC18 *ori*V_{Ec})	[7]

pEC-XC99E	Cm^R; *C. glutamicum*/*E. coli* shuttle plasmid for regulated gene expression using P_{trc}(P_{trc} *lacI*^{q} pGA1 *oriV*$_{Cg}$, *oriV*$_{Ec}$)	[8]
pEC-P_{tetR}	Cm^R; *C. glutamicum*/*E. coli* shuttle plasmid for regulated gene expression using P_{tetR}(pGA1 *oriV*$_{Cg}$, *oriV*$_{Ec}$)	This work
pMKEx1	Kan^R; *C. glutamicum*/*E. coli*/ shuttle vector based on pJC1 for expression of target genes under control of the T7 promoter (P*lacI*, *lacI*, PT7, *lacO1*, N-term. strep tagII, MCS, C-term. His tag, pHM1519 *oriCg*; pACYC177 *oriEc*)	[6]
pVWEx2	Tet^R; *C. glutamicum*/ *E. coli* shuttle vector for regulated gene expression; (Ptac, *lacIQ*, pCG1 *oriVC.g.*, pUC18 *oriV E.c*.)	[9]
pET3a_*pduABJKNUT* (pJP063)	Amp^R; overexpression vector with T7 promoter for expression of seven shell genes	[10]
pET14b-pdu65 (pED460)	Amp^R; cloning vector containing *pduA, pduB, pduC, pduD, pduE, pduG, pduH, pduJ, pduK, pduL, pduM, pduN, pduO, pduP, pduQ, pduS, pduT, pduX*	[2]
pEKEx2_eyfp$_{asv}$	Kan^R; P_{tac}; overexpression of *eyfp*, includes gene sequence for the C-terminal *C. glutamicum* SsrA tag variation AAEKSQRDYAASV	[11]
pAN6_*pduA-X*	Kan^R; P_{tac}; overexpression vector for production of all *pdu* genes (as present on pET14b-pdu65)	This work
pMKEx1_*pduABJKNUT*	Kan^R; P_{T7}; overexpression vector for production of all *pdu* shell genes	This work
pMKEx1_*PduA*	Kan^R; P_{T7}; overexpression vector for production of PduA	This work
pMKEx1_*pduABJKNTU*$_{native}$	Kan^R; P_{T7}; overexpression vector for expression of the native *pduABJKNTU* operon	This work
pMKEx1_*pduABJKN*	Kan^R; P_{T7}; overexpression vector for production of PduABJKN	This work
pMKEx1_*pduABJKNU*	Kan^R; P_{T7}; overexpression vector for production of PduABJKNU	This work
pMKEx1_*pduABJkN**	Kan^R; P_{T7}; overexpression vector for production of PduABJkN	This work
pMKEx1_*pduABJkNu**	Kan^R; P_{T7}; overexpression vector for production of PduABJkNu	This work
pMKEx1_*pduABJkNut**	Kan^R; P_{T7}; overexpression vector for production of PduABJkNut	This work
pMKEx1_*pduABJkn**	Kan^R; P_{T7}; overexpression vector for production of PduABJkn	This work
pMKEx1_*pduABJknt**	Kan^R; P_{T7}; overexpression vector for production of PduABJknt	This work
pMKEx1_*pduABJkNt**	Kan^R; P_{T7}; overexpression vector for production of PduABJkNt	This work
pMKEx1_*pduABJkn**	Kan^R; P_{T7}; overexpression vector for production of PduABJkn	This work
pMKEx1_*pduJ*	Kan^R; P_{T7}; overexpression vector for production of PduJ	This work
pMKEx1_*pduABJKnut**	Kan^R; P_{T7}; overexpression vector for production of PduABJKnut	This work
pMKEx1_*pduABJKnt**	Kan^R; P_{T7}; overexpression vector for production of PduABJKnt	This work
pMKEx1_*pduA*$_{PDZlig}$	Kan^R; P_{T7}; overexpression vector for production of PduA C-terminally tagged with PDZligand (GGCGTGAAGGAATCCCTGGTG); Linker: GGATCTGGTTCCGGCTCCGGTTCCGGC [$(GS)_4G$]	This work
pMKEx1_*pduA*$_{SH3lig}$	Kan^R; P_{T7}; overexpression vector for production of PduA C-terminally tagged withSH3ligand (CCACCACCAGCACTGCCACCAAAGCGCCGCCGC); Linker: [$(GS)_4G$]	This work
pMKEx1_*pduA*$_{GBDlig}$	Kan^R; P_{T7}; overexpression vector for production of PduA C-terminally tagged with GBDligand (Sequence S1); Linker: [$(GS)_4G$]	This work
pMKEx1_*pduA*$_{PDZlig}$*BJknt**	Kan^R; P_{T7}; overexpression vector for production of PduABJknt with PduA C-terminally tagged with PDZligand	This work
pMKEx1_*pduA*$_{SH3lig}$*BJknt**	Kan^R; P_{T7}; overexpression vector for production of pduABJknt with PduA C-terminally tagged with SH3ligand	This work
pMKEx1_*pduA*$_{GBDlig}$*BJknt**	Kan^R; P_{T7}; overexpression vector for production of PduABJknt with PduA C-terminally tagged with GBDligand	This work
pK19*mobSacB*	Kan^R; vector for allelic exchange in *C. glutamicum* (oriT oriV$_{Ec}$ *sacB lacZ*α)	[12]
pK19_CGP1int_P_{T7}-*eyfp*	Kan^R; pK19*mobSacB* derivative for allelic integration of *eyfp* under control of P_{T7} into CGP1 region	Meike Baumgart
pK19_*pduABJKNUT*	Kan^R; Derivative of pK19-CGP1int_P_{T7}-*eYFP*, *eYFP* was exchanged with *pduABJKNUT*	This work

pK19_*pduABJknt**	Kan^R; Derivative of pK19-CGP1int_P_{T7}-*eYFP*, *eYFP* was exchanged with *pduABJknt**	This work
pK19_*pduA*	Kan^R; Derivative of pK19-CGP1int_P_{T7}-*eYFP*, *eYFP* was exchanged with *pduA*	This work
pEC_*P18eyfp*	Cm^R; derivative of pEC_$P_{tetR;}$ regulated expression of *eyfp* tagged with P18 targeting peptide (ATGAACACTTCAGAACTTGAAACCCTTATTCG TAACATTTTGAGTGAGCAACTT); Linker: AGATCT [BglII]	This work
pEC_*P18eyfp*$_{asv}$	Cm^R; derivative of pEC_P_{tetR}; regulated expression of *eyfp* tagged with P18 targeting peptide and asv degradation peptide, linker: [BglII]; Asv tag (GCAGCAGAAAAGAGCCAACGTGATTACGCTGCATCAGTT)	This work
pEC_*D18eyfp*	Cm^R; derivative of pEC_P_{tetR}; regulated expression of *eyfp* tagged with D18 targeting peptide (ATGGAAATCAATGAAAAGCTGCTGCGCCAGATTATTGAAGACGTA CTGTCTGAA); linker: [BglII]	This work
pEC_*D18eyfp*$_{asv}$	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with D18 targeting peptide and asv degradation peptide; linker: [BglII]	This work
pEC_*D18eyfp_P18cfp*	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with D18 targeting peptide and *cfp* tagged with P18 targeting peptide; Linker: AGATCT [BglII]	This work
pEC_*eyfp*$_{PDZdom}$	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with C-terminal *PDZdomain* (Sequence S1)*;* Linker: GGATCTGGTTCCGGCTCCGGTTCCGGC [$(GS)_4G$]	This work
pEC_*eyfp*$_{SH3dom}$	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with C-terminal *SH3domain* (Sequence S1)*;* Linker: [$(GS)_4G$]	This work
pEC_*eyfp*$_{GBDdom}$	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with C-terminal *GBDdomain* (Sequence S1)*;* Linker: [$(GS)_4G$]	This work
pEC_*eyfpC18*$_{K.p.}$	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with C-terminal targeting peptide from *K. pneumonia* (AACGAACAGAACGTGGA ACGCGTGATCCGCCAGGTGCTGGAACGCCTGGCAAAG) Linker: GGCGGTGGCTCCGGCGGCGGTTCCGGCGGT [$(GGGS)_2GG$]	This work
pEC_*eyfpC18*$_{P.m.}$	Cm^R; P_{tetR}; regulated expression of *eyfp* tagged with C-terminal targeting peptide from *P. mirabilis;* Linker: [$(GGGS)_2GG$]; C18 peptide (ACCGAAGAAAACGTGGAACGCATCATCAAGGAAGTGCTGGGCCG CCTGGGCAAG)	This work
pEC_*eyfp-P18*	Cm^R; P_{tetR}; regulated expression of *eyfp* C-terminally tagged with P18 targeting peptide; linker: [$(GGGS)_2GG$]	This work
pVWEx2-*pdc*	Tet^R; derivative of pVWEx2, expression of *pdc* from *Z. mobilis* under control of constitutive P_{tuf} promoter	This work
pVWEx2-*P18-NdeI-pdc*	Tet^R; P_{tuf}*; P18pdc* expression; Linker: GAATTC [NdeI]	This work
pVWEx2-*P18-GS-pdc*	Tet^R; P_{tuf}*; P18pdc* expression ; Linker GGTTCT [GS]	This work
pVWEx2-*P18-GSGS-pdc*	Tet^R; P_{tuf}*; P18pdc* expression; Linker: GGTTCTGGCTCC [GSGS]	This work
pVWEx2-*adhB*	Tet^R; derivative of pVWEx2, expression of *adhB* from *Z. mobilis* under control of constitutive P_{tuf} promoter	This work
pVWEx2-*D18-GSGS-adhB*	Tet^R; P_{tuf}*; D18adhB* expression; Linker: GGTTCTGGCTCC [GSGS]	This work
pVWEx2-*D18-10aa-adhB*	Tet^R; P_{tuf}*; D18adhB* expression; Linker: AGGGCTCTGGATCGACATCAGGCTCCGGT [10 aa]	This work
pVWEx2-*D60-adhB*	Tet^R; P_{tuf}*; D60adhB* expression; No linker	This work
pVWEx2_*adhB-GBD*$_{dom}$	Tet^R; P_{tuf}*; adhB-GBD*$_{dom}$ expression; Linker: GGATCTGGTTCCGGCTCCGGTTCCGGC [$(GS)_4G$]	This work
pVWEx2_*adhB-SH3*$_{dom}$	Tet^R; P_{tuf}*; adhB-SH3*$_{dom}$ expression; Linker: [$(GS)_4G$]	This work
pVWEx2_*adhB-PDZ*$_{dom}$	Tet^R; P_{tuf}*; adhB-PDZ*$_{dom}$ expression; Linker: [$(GS)_4G$]	This work
pVWEx2-P_{tuf}*adhB_pdc*	Tet^R; P_{tuf}*; adhB* and *pdc* expression	This work
pVWEx2-*D18-GSGS-adhB_P18pdc*	Tet^R; P_{tuf}*; D18adhB* and *P18pdc* expression; Linker between *D18* and *adhB*: [GSGS]; Linker between *P18* and *pdc*: [NdeI]	This work
pVWEx2-*D18-10aa-adhB_P18pdc*	Tet^R; P_{tuf}*; D18adhB* and *P18pdc* expression; Linker between *D18* and *adhB*: [10aa]; Linker between *P18* and *pdc*: [NdeI]	This work

Table S2: Number of cells with ‚BMC-like‘ structures. Cells were counted to contain ‘BMC’-like if they contained at least 1 closed BMC-like structure.

Strain	Cells with ‘BMC-like‘	No BMCs	Total	% of cells with ‘BMC’-like
MB001(DE3) *pduABJKNUT*	8	192	200	4%
MB001(DE3) *pduA$_{PDZlig}$BJknt*	115	85	200	58%
MB001(DE3) *pduA$_{GBDlig}$BJknt*	68	132	200	34%
MB001(DE3) *pduA$_{SH3lig}$BJknt*	45	155	200	23%
MB001(DE3) *pduABJknt*	91	109	200	46%
MB001(DE3) *pduABJkNt*	76	124	200	38%
MB001(DE3) *pduABJKnut*	12	188	200	6%
MB001(DE3) *pduABJkNut*	105	95	200	53%
MB001(DE3) *pduABJkn*	51	149	200	26%
MB001(DE3) *pduABJkN*	57	143	200	29%
MB001(DE3)::P$_{T7}$*pduABJknt*	38	162	200	19%
MB001(DE3)	7	193	200	4%

Table S3: Amino acid distribution of different C- and N-terminal targeting peptides. Adapted from [13].

	Aliphatic I,L,V	Aromatics F,W,Y	Hydrophylic K,R, D,E, Q, N	Tiny G,A,S	H	C	M,T	P
AldhDH_C17, *Klebsiella pneumoniae*								
NEQNVERVIRQVLERLA	35.3%	0.0%	58.8%	5.9%	0.0%	0.0%	0.0%	0.0%
AldhDH_C17, *Proteus mirabilis*								
TEENVERIIKEVLGRLG	35.3%	0.0%	47.1%	11.8%	0.0%	0.0%	5.9%	0.0%
PduD(2-18), *Citrobacter freundii*								
NEKLLRQIIEDVLSEMQ	35.3%	0.0%	52.9%	5.9%	0.0%	0.0%	5.9%	0.0%
PduP(2-18), *Citrobacter freundii*								
NTSELETLIRNILSEQL	35.3 %	0.0 %	41.1 %	11.8%	0.0%	0.0%	11.8%	0.0%

Table S4: Construction of Pdu production plasmids. Numbers represent oligonucleotide pairs used for PCR (Table S7). The DNA template used for amplification is given in brackets behind the oligonucleotides followed by the information on the plasmid backbone and the restriction enzymes used for linearization.

Plasmids	Construction
pAN6_*pduA-X*	Gibson assembly: 725/726 and 727/728 (pED460) and pED460 *AscI *KpnI into pAN6 *NdeI *EcoRI
pAN6_*pduABJKNUT*	Gibson assembly: 725/729 (pET3a_*pduABJKNUT*) into pAN6 *NdeI *EcoRI
pMKEx1_*pduABJKNUT*	Gibson assembly: 036/028 (pAN6_*pduABJKNUT*) into pMKEx1 *BamHI *NcoI
pMKEx1_*PduA*	Gibson assembly: 140/159 (pMKEx1_*pduABJKNUT*) into pMKEx1 *BamHI * XbaI
pMKEx1_*pduABJKNTU$_{native}$*	Gibson assembly: 140/178 (pMKEx1_*pduABJKNUT*), 179/180, 181/182 and 183/174 (pAN6_*pduA-X*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJkN*	Gibson assembly: 140/168 and 167/176 (pMKEx1_*pduABJKNUT*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJkNu*	Gibson assembly: 140/168, 167/170 and 169/174 (pMKEx1_*pduABJKNUT*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJkNut*	Gibson assembly: 140/168,167/170, 169/172, 171/028 (pMKEx1_*pduABJKNUT)* into pMKEx1 *XbaI *BamHI

pMKEx1_*pduABJkn*	Gibson assembly: 140/168neu 167neu/200 199/176 (pMKEx1_*pduABJkNut*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJknt*	Gibson assembly: 140/168neu, 167neu/200, 199/202 and 201/028 (pMKEx1_*pduABJkNut*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJkNt*	Gibson assembly: 140/168neu, 167neu/202 and 201/028 (pMKEx1_*pduABJkNut*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduJ*	Gibson assembly: 232/184 (pMKEx1_*pduABJKNUT*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJKnut*	Gibson assembly: 140/200 (pMKEx1_*pduABJKNUT)* and 199/028 *(*pMKEx1_*pduABJkNut)* into pMKEx1 *XbaI *BamHI
pMKEx1_*pduABJKnt*	Gibson assembly: 140/200 (pMKEx1_*pduABJKNUT*) and 199/028 (pMKEx1_*pduABJknt)* into pMKEx1 *XbaI *BamHI
pMKEx1_*pduA$_{GBDlig}$BJKNUT*	Gibson assembly: 137/138 (Protein_scaffolds$_{opt}$), 136/726 and 139/140 (pMKEx1_*pduABJKNUT*) into pMKEx1_*PduABJKNUT* *AscI * XbaI
pMKEx1_ *pduA$_{SH3lig}$BJKNUT*	Gibson assembly: 139/140 and 142/726 (pMKEx1_*pduABJKNUT*) into pMKEx1_*PduABJKNUT* *AscI *XbaI
pMKEx1_ *pduA$_{PDZlig}$BJKNUT*	Gibson assembly: 139/140 and 141/726 (pMKEx1_*pduABJKNUT*) into pMKEx1_*PduABJKNUT* *AscI *XbaI
pMKEx1_*pduA$_{PDZlig}$BJknt*	Gibson assembly: 240/028 (pMKEx1_*pduABJknt*) and 140/242 (pMKEx1_*pduA$_{PDZlig}$BJKNUT*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduA$_{SH3lig}$BJknt*	Gibson assembly: 243/140 *pduA$_{SH3lig}$BJKNUT* and 240/028 (pMKEx1_*pduABJknt*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduA$_{GBDlig}$BJknt*	Gibson assembly: 241/140 (pMKEx1_*pduA$_{GBDlig}$BJKNUT)* and 240/028 (pMKEx1_*pduABJknt*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduA$_{PDZlig}$*	Gibson assembly: 140/156 (pMKEx1_*pduA$_{PDZlig}$BJknt*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduA$_{SH3lig}$*	Gibson assembly: 140/157 (pMKEx1_*pduA$_{SH3Zlig}$BJknt*) into pMKEx1 *XbaI *BamHI
pMKEx1_*pduA$_{GBDlig}$*	Gibson assembly: 140/158 (pMKEx1_*pduA$_{GBDlig}$BJknt*) into pMKEx1 *XbaI *BamHI
pK19_*pduABJknt*	Gibson assembly: 197/198 (pMKEx1_*pduABJknt*) into pK19_CGP1int_P$_{T7}$*eyfp* *XbaI *BlpI
pK19_*pduA*	Gibson assembly: 197/198 (pMKEx1_*pduA*) into pK19_CGP1int_P$_{T7}$*eyfp* *XbaI *BlpI

Table S5: Construction of different fluorescence reporter production plasmids. Numbers represent oligonucleotide pairs used for PCR (Table S7). The DNA template used for amplification is given in brackets behind the oligonucleotides followed by the information on the plasmid backbone and the restriction enzymes used for linearization.

Plasmid	**Construction**
pEC-P$_{tetR}$	Gibson assembly: 780/012 (pCL-TON1) into pEC-XC99E *PstI *NdeI
pEC_*eyfp*	Cloning: 008/009 (pEKEx2_eyfp$_{asv}$)*BcuI *EcoRI ligated into pEC-TetR *BcuI * EcoRI
pEC_*P18*	Cloning: 003/004 (pED460)*BglII *EcoRV ligated into pEC-TetR *BglII *EcoRV
pEC_*D18*	Cloning: 005/006 (pED460)*BglII *EcoRV ligated into pEC-TetR *BglII *EcoRV
pEC_*P18eyfp*	Cloning: 007/013 (pEKEx2_eyfp$_{asv}$) *BglII *BcuI into pEC_*P18* *BglII *BcuI
pEC_*P18eyfp$_{asv}$*	Cloning: 007/014 (pEKEx2_eyfp$_{asv}$) *BglII *BcuI into pEC_*P18* *BglII *BcuI
pEC_*D18eyfp*	Cloning: 007/013 (pEKEx2_eyfp$_{asv}$) *BglII *BcuI into pEC_*D18* *BglII *BcuI
pEC_*D18eyfp$_{asv}$*	Cloning: 007/014 (pEKEx2_eyfp$_{asv}$) *BglII *BcuI into pEC_*D18* *BglII *BcuI
pEC_*D18eyfp_P18cfp*	Gibson assembly: 215/217 (pEC_*D18eyfp),* 216/218 (*cfp*) into pEC_*P18eyfp* *BcuI
pEC_eyfp$_{PDZdom}$	Gibson assembly: 143/144 (Protein_scaffolds$_{opt}$) into pEC_eyfp *BcuI
pEC_eyfp$_{SH3dom}$	Gibson assembly: 145/146 (Protein_scaffolds$_{opt}$) into pEC_eyfp *BcuI
pEC_eyfp$_{GBDdom}$	Gibson assembly: 147/148 (Protein_scaffolds$_{opt}$) into pEC_eyfp *BcuI

pEC_*eyfp-P18*	Gibson assembly: 109/114 (pEC_*eyfp*) and 116/115 (pEC-*P18eyfp*) into pEC_TetR *EcoRV *BcuI
pEC_*eyfp-C17*$_{K.p.}$	Gibson assembly: 109/131 (pEC_*eyfp(GGGS)2GG_P18*) and 109/132 (PCR product 109/131) into pEC_TetR *EcoRV *BcuI
pEC_*eyfp-C17*$_{P.m.}$	Gibson assembly: 109/114 (pEC_*eyfp*) and 134/135 (Protein_scaffolds$_{opt}$) into pEC_TetR *EcoRV *BcuI

Table S6: Construction of AdhB and Pdc production plasmids. Numbers represent oligonucleotide pairs used for PCR (**Fehler! Verweisquelle konnte nicht gefunden werden.**). The DNA template used for amplification is given in brackets behind the oligonucleotides followed by the information on the plasmid backbone and the restriction enzymes used for linearization.

Plasmid	**Construction**
pVWEx2_*pdc*	Gibson assembly: K15/K16 (*Z. mobilis* genome) into pVWEx2 *XbaI *SalI
pVWEx2_*P18-NdeI-pdc*	Gibson assembly: K24/K16 (*Z. mobilis* genome) and K27/K28 (pEC_*P18eyfp*) into pVWEx2 *XbaI *SalI
pVWEx2_*P18-GS-pdc*	Gibson assembly: K26/K16 (*Z. mobilis* genome) and K30/K27 (pEC_*P18eyfp*) into pVWEx2 *XbaI *SalI
pVWEx2_*P18-GSGS-pdc*	Gibson assembly: K25/K16 (*Z. mobilis* genome) and K29/K27 (pEC_*P18eyfp*) into pVWEx2 *XbaI *SalI
pVWEx2_*adhB*	Gibson assembly: K14/K13 (*Z. mobilis* genome) into pVWEx2 *XbaI *SalI
pVWEx2_*D18-GSGS-adhB*	Gibson assembly: K14/K19 (*Z. mobilis* genome) and K34/K23 (pEC_*D18eyfp*) into pVWEx2 *XbaI *SalI
pVWEx2_*D18-10aa-adhB*	Gibson assembly: K35/K14 (*Z. mobilis* genome) and K20/K36 (pVWEx2_*D18-GSGS-adhB*) into pVWEx2 *XbaI *SalI
pVWEx2_*D60-adhB*	Gibson assembly: K33/K14 (*Z. mobilis* genome) and K34/K32 (pET14b-pdu65) into pVWEx2 *XbaI *SalI
pVWEx2_*adhB-GBD*$_{dom}$	Gibson assembly: K13/257 (pVWEx2-*adhB*) and 258/260 (pEC_*eyfp*$_{GBDdom}$) into pVWEx2 *SalI*XbaI
pVWEx2_*adhB-SH3*$_{dom}$	Gibson assembly: K13/257 (pVWEx2_*adhB*) and 258/261 (pEC_*eyfp*$_{SH3dom}$) into pVWEx2 *SalI*XbaI
pVWEx2_*adhB-PDZ*$_{dom}$	Gibson assembly: K13/257 (pVWEx2_*adhB*) and 258/259 (pEC_*eyfp*$_{PDZdom}$) into pVWEx2 *SalI*XbaI
pVWEx2-P$_{tuf}$*adhB_pdc*,	Gibson assembly: K38/K16 (pVWEx2_*pdc)* and K13/K39 (pVWEx2-*adhB)* into pVWEx2 *SalI*XbaI
pVWEx2-P$_{tuf}$*D18-GSGS-adhB_P18pdc*	Gibson assembly: K20/K39 (pVWEx2_*D18-GSGS-adhB*) and K37/K16 (pVWEx2_*P18-NdeI-pdc*) into pVWEx2 *SalI*XbaI
pVWEx2-P$_{tuf}$*D18-10aa-adhB_P18pdc*	Gibson assembly: K20/K39 (pVWEx2-*D18-10aa-adhB*) and K37/K16 (pVWEx2_*P18-NdeI-pdc*) into pVWEx2 *SalI*XbaI

Table S7: Oligonucleotides used in this study.

Oligonucleotide	**Sequence (5' → 3')**
(003)	ACGTGATATCCATATGAACACTTCAGAACTTGA
(004)	ACGTAGATCTAAGTTGCTCACTCAAAATGT
(005)	ACGTGATATCCATATGGAAATCAATGAAAAGCT
(006)	ACGTAGATCTTTCAGACAGTACGTCTTCAA
(007)	ACGTAGATCTGTGAGCAAGGGCGAGGAGCT

Oligonucleotide	Sequence (5’ → 3’)
(008)	ACGTGATATCCATATGGTGAGCAAGGGCGAGGA
(012)	CCAAGCTTGCATGCCTGCAGTTAACTAGTTCCAGATCTTCCCATATGGATATCTCCTTGTGTATCAACAAGCTGGGGATC
(013)	ACGTACTAGTTCTAGACTTGTACAGCTCGT
(014)	ACGTACTAGTTTAAACTGATGCAGCGTAATCAC
(028)	CGGAGCTCGAATTCGGATCCTTATCCCTCCACCATCTGTC
(036)	CTTTAAGAAGGAGATATACCATGCAACAAGAAGCGTTAGG
(109)	GTTGATACACAAGGAGATATCCATATGGTGAGCAAGGGCGAGGAGC
(114)	GCCGCCGGAGCCACCGCCTCTAGACTTGTACAGCTCGTCCATGC
(115)	CGGTGGCTCCGGCGGCGGTTCCGGCGGTATGAACACTTCAGAACTTGAAACCC
(116)	CATGCCTGCAGTTAACTAGTTTAAAGTTGCTCACTCAAAATG
(131)	GTTCCAGCACCTGGCGGATCACGCGTTCCACGTTCTGTTCGTTACCGCCGGAACCGCCGCCG
(132)	CTTGCATGCCTGCAGTTAACTAGTTTACTTTGCCAGGCGTTCCAGCACCTGGCGGATCACG
(134)	GACGAGCTGTACAAGTCTAGAGGCGGTGGCTCCGGCGGCGGTTC
(135)	AGCTTGCATGCCTGCAGTTAACTAGTTTACTTGCCCAGGCGGCCCAGCAC
(136)	AGATGAAGATTAATGAGCAGCAATGAGCTGGTTG
(137)	TCAACCAGCTCATTGCTGCTCATTAATCTTCATCTTCATCGC
(138)	GGATCTGGTTCCGGCTCCGGTTCCGGCCTGGTGGGCGCACTGATGCAC
(139)	GCCGGAACCGGAGCCGGAACCAGATCCGCTAATTCCCTTCGGTAAG
(140)	GTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAAC
(141)	GTTCCGGCTCCGGTTCCGGCGGCGTGAAGGAATCCCTGGTGTAATGAGCAGCAATGAGCTGG
(142)	GTTCCGGCTCCGGTTCCGGCCCACCACCAGCACTGCCACCAAAGCGCCGCCGCTAATGAGCAGCAATGAGCTG
(143)	TTGCATGCCTGCAGTTAACTAGTTTACTTGAAATAGGGTGAGACCTCCTTCATG
(144)	CGAGCTGTACAAGTCTAGAACTAGTGGATCTGGTTCCGGCTCCGGTTCCGGCCTCCAGCGTCGCCGCGTGAC
(145)	GCTTGCATGCCTGCAGTTAACTAGTTTAGCGATACTTCTCCACGTAAGGGACAG
(146)	GAGCTGTACAAGTCTAGAACTAGTGGATCTGGTTCCGGCTCCGGTTCCGGCGCAGAGTATGTGCGTGCCCTC
(147)	GCATGCCTGCAGTTAACTAGTTTATGGTGCTTGGCGACGGAGTTCATTTTTAAC
(148)	ACGAGCTGTACAAGTCTAGAACTAGTGGATCTGGTTCCGGCTCCGGTTCCGGCACCAAGGCAGATATTGGAAC
(156)	CTTGTCGACGGAGCTCGAATTCGGATCCTTACACCAGGGATTCCTTCAC
(157)	GTCGACGGAGCTCGAATTCGGATCCTTAGCGGCGGCGCTTTGGTG
(158)	AGCTTGTCGACGGAGCTCGAATTCGGATCCTTAATCTTCATCTTCATCG
(159)	CTTGTCGACGGAGCTCGAATTCGGATCCTTAGCTAATTCCCTTCGGTAAG
(167)	AGTGGTGAAGCAATCACTGGGATTACTTGAAGTTAGTGGTC
(168)	ACTTCAAGTAATCCCAGTGATTGCTTCACTTGTATATCTCCTTCTTAAAG
(169)	AGTGGAAAGACAACCCACCACGGATCGTATGATTCAG
(170)	GTGGTGGGTTGTCTTTCCACTTGTATATCTCCTTCTTAAAG
(171)	AGTGTCTCAGGCTATAGGGATTTTAGAAC
(172)	CCCTATAGCCTGAGACACTTTATGTATATCTCCTTCTTAAAG
(174)	TCGACGGAGCTCGAATTCGGATCCTTATGTCCGGGTGATGGGAC
(176)	TCGACGGAGCTCGAATTCGGATCCCTAACGAGAAAGCGTGTCGAC
(178)	ATACTGCTTTTCTCCTGTGGGTCAGATGTAGGACGGACGATC

Oligonucleotide	Sequence (5’ → 3’)
(179)	ATCGTCCGTCCTACATCTGACCCACAGGAGAAAAGCAGTATG
(180)	ACCCGTGCCAGATGCATAGCTCACGCTTCACCTCGTTTGC
(181)	GCAAACGAGGTGAAGCGTGAGCTATGCATCTGGCACGGGTTAC
(182)	CTATAGCCTGAGACATGACTAACGAGAAAGCGTGTCGACAATG
(183)	CATTGTCGACACGCTTTCTCGTTAGTCATGTCTCAGGCTATAG
(184)	TGTCGACGGAGCTCGAATTCGGATCCTTATGCGGATTTAGGTAAAATG
(197)	GTGAGCGGATAACAATTCCCCTCTAG
(198)	GCCCCAAGGGGTTATGCTAGTTATTGCTCAG
(199)	CTTTAAGAAGGAGATATACATTTGCATCTGGCACGGGTTAC
(200)	GTAACCCGTGCCAGATGCAAATGTATATCTCCTTCTTAAA
(201)	TTAAGAAGGAGATATACAAGTGTCTCAGGCTATAGGGATT
(202)	AATCCCTATAGCCTGAGACACTTGTATATCTCCTTCTTAAAG
(215)	CGAGCTGTACAAGTCTAGAACTAGTTAATTAAGATCCCCAGCTTGTTG
(216)	TGAAGACGTACTGTCTGAAAGATCTGTGAGCAAGGGCGAGGAGCTG
(217)	ACAGCTCCTCGCCCTTGCTCACAGATCTTTCAGACAGTACGTCTTC
(218)	CTTGCATGCCTGCAGTTAACTAGTTTACTTGTACAGCTCGTCCATG
(232)	GTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAAC
(240)	ATTTTGTTTAACTTTAAGAAGGAGATATACATATGAGCAGCAATGAGCTGGTTGATC
(241)	CTTCTTAAAGTTAAACAAAATTATTTCTAGTTTAATCTTCATCTTCATCGCCTG
(242)	CTTCTTAAAGTTAAACAAAATTATTTCTAGTTACACCAGGGATTCCTTCAC
(243)	TTCTTAAAGTTAAACAAAATTATTTCTAGTTAGCGGCGGCGCTTTGGTGGC
(257)	ACCGGAGCCGGAACCAGATCCGAAAGCGCTCAAGAAGAGTTC
(258)	AAGAACTCTTCTTGAGCGCTTTCGGATCTGGTTCCGGCTCCG
(259)	GTACCCGGGGATCCTCTAGATTACTTGAAATAGGGTGAGAC
(260)	TACCCGGGGATCCTCTAGATTATGGTGCTTGGCGACGGAGTTC
(261)	GTACCCGGGGATCCTCTAGATTAGCGATACTTCTCCACGTAAGG
(725)	CTGCAGAAGGAGATATACATATGCAACAAGAAGCGTTAGGAATGG
(726)	ATCAGGACACCAACGGATGCCGG
(727)	TTCGTCGTTATGGTTTTCATGGTACC
(728)	GTAAAACGACGGCCAGTGAATTCGACCCTTATTGCAGTTCGACC
(729)	GTAAAACGACGGCCAGTGAATTCTTATCCCTCCACCATCTGTCG
(780)	GTGCGGTATTTCACACCGCAGCTTTTAAGACCCACTTTCACATTTAAG
(K13)	GTCGTAGCCACCACGAAGTCCGTCGACGAAAGGAGGTCTATATGGCTTCTTCAACTTTTTATATTC
(K14)	GCTCGGTACCCGGGGATCCTCTAGATTAGAAAGCGCTCAAGAAGAGTTC
(K15)	CGTAGCCACCACGAAGTCCGTCGACGAAAGGAGGTCTATATGAGTTATACTGTCGGTAC
(K16)	TCGGTACCCGGGGATCCTCTAGACTAGAGGAGCTTGTTAACAGG
(K19)	CGTACTGTCTGAAGGTTCTGGCTCCGCTTCTTCAACTTTTTATATTCCTTTCG
(K20)	CGTAGCCACCACGAAGTCCGTCGACGAAAGGAGGTCTATATGG
(K23)	GAATATAAAAAGTTGAAGAAGCGGAGCCAGAACCTTCAGACAGTACGTCTTCAATAATC
(K24)	GCAACTTCATATGAGTTATACTGTCGGTACCTATTTAG
(K25)	ACTTGGTTCTGGCTCCAGTTATACTGTCGGTACCTATTTAG

Oligonucleotide	Sequence (5' → 3')
(K26)	GCAACTTGGTTCTAGTTATACTGTCGGTACCTATTTAGC
(K27)	GTCGTAGCCACCACGAAGTCCGTCGACGAAAGGAGGTCTATATGAACACTTCAGAACTTGAAACC
(K28)	GTACCGACAGTATAACTCATATGAAGTTGCTCACTCAAAATGTTAC
(K29)	GTACCGACAGTATAACTGGAGCCAGAACCAAGTTGCTCACTCAAAATGTTACG
(K30)	ATAGGTACCGACAGTATAACTAGAACCAAGTTGCTCACTCAAAATG
(K32)	AAGTTGAAGAAGCTTGCTGCTGGCCTTGTTTGGCTTCGCCAATC
(K33)	GCCAAACAAGGCCAGCAGCAAGCTTCTTCAACTTTTTATATTCC
(K34)	CGTAGCCACCACGAAGTCCGTCGACGAAAGGAGGTCTATATGGAAATCAATGAAAAGCTGCTG
(K35)	CTGGATCGACATCAGGCTCCGGTGCTTCTTCAACTTTTTATATTCCTTTCGTCAAC
(K36)	GGAGCCTGATGTCGATCCAGAGCCCTTTTCAGACAGTACGTCTTCAATAATCTG
(K37)	ATTGATGCGGAAAGGAGGTCTATATGAACACTTCAGAACTTGAAACCCTTATTCG
(K38)	ATGCGGAAAGGAGGTCTATATGAGTTATACTGTCGGTACCTATTTAGC
(K39)	CATATAGACCTCCTTTCCGCATCAATCATTAGAAAGCGCTCAAGAAGAG

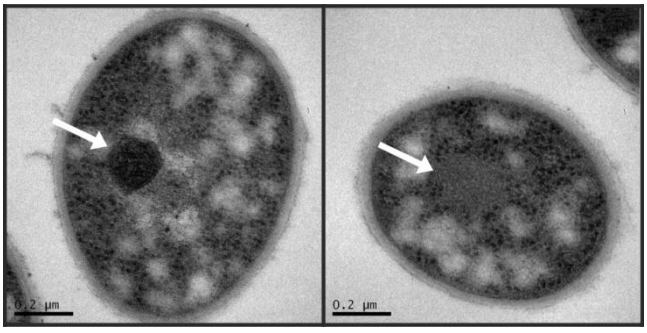

Figure S1: Transmission electron microscopy of *C. glutamicum* MB001(DE3). Arrow in the left image marks volutin granule [14] and arrow within the right image an unknown artefact.

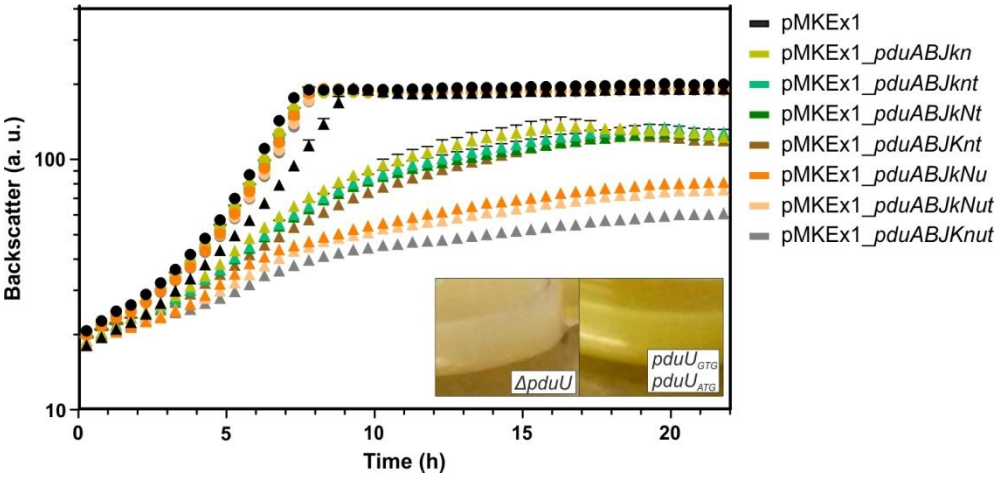

Figure S2: Growth of different *C. glutamicum* MB001(DE3) Pdu production strains. A Growth in CgXII + 2% (w/v) glucose. Triangles: Induction with 50 µM IPTG; Circles: 0 µM IPTG;

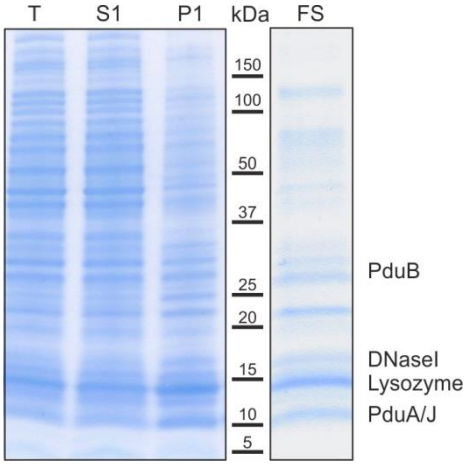

Figure S3: PduABJknt purification approach. T: Cell lysate purified from *C. glutamicum* MB001(DE3) *pduABJknt* in YPER plus after centrifugation at 4000 *g.* S1: Supernatant after centrifugation at 11,000 *g.* P1: Pellet after centrifugation at 11,000 *g.* FS: Final supernatant after BMC precipitation with 160 mM NaCl and final clarification step with centrifugation step at 11,000 *g* for 5 min.

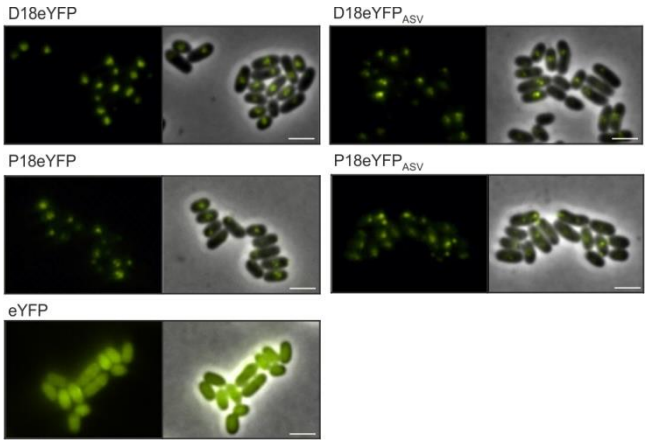

Figure S4: Fluorescence microscopy analysis of different *C. glutamicum* MB001(DE3)::P$_{T7}$*PduABJknt* strains. BMC production was induced with 250 µM IPTG after 2 h of cultivation. After induction, the cells were cultivated at 30°C for 4 h. Scale bar is 2 µm.

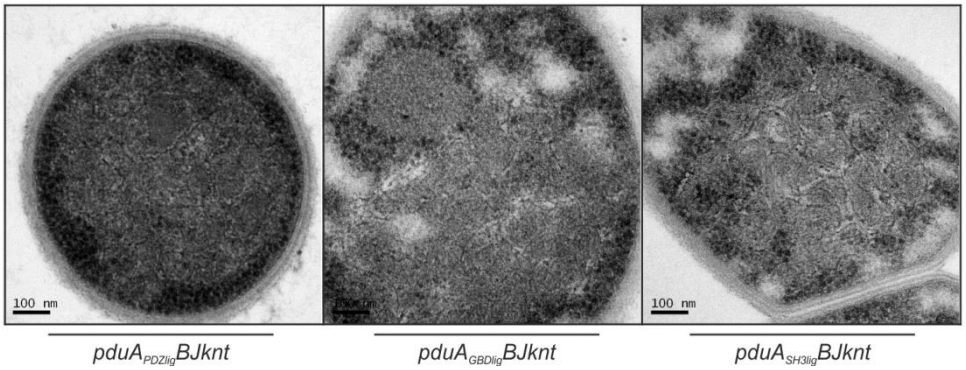

Figure S5: *C. glutamicum* MB001(DE3*) pduA$_{lig}$BJknt* strains with irregularly shaped BMCs. BMC production was induced with 50 µM IPTG and cells were further cultivated for 4 h.

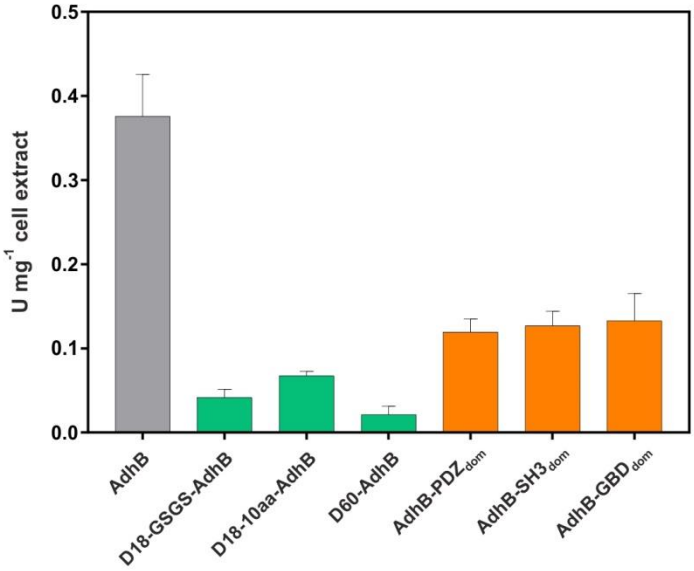

Figure S6: Activity measurements of AdhB versions in crude cell extracts. As proof-of-principle, we wanted to examine the enzyme activities of alcohol dehydrogenase (AdhB) versions tagged the synthetic C-terminal interaction domain SH3, GBD and PDZ. The constitutive P_{tuf} promoter was used for the production of the enzymes in MB001(DE3) to achieve a moderate expression. The untagged AdhB version showed an activity of 0.376 U mg^{-1} cell extract. All C-terminal tagged AdhB versions (AdhB-GBD_{dom}, AdhB-PDZ_{dom}, AdhB-$SH3_{dom}$) have a similarly reduced activity with 0.119, 0.127 and 0.133 U mg^{-1} cell extract and, thus, showed a 2-fold higher activity than the best D18-AdhB version. In comparison to the untagged AdhB version, the AdhB-domain versions maintain 30% of the activity. The C-terminal targeting was proven to enhance enzyme activity for AdhB in comparison to the N-terminally tagged versions and provide a novel alternative for enzyme targeting into BMCs. We also assume that the enhancement of activity with C-terminal targeting can be transferred to other metabolic enzymes.

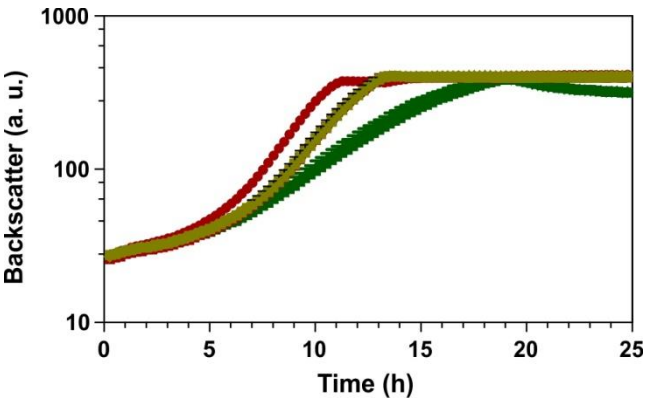

Figure S7: Aerobic cultivation of ethanol production strains. MB001(DE3) (red, circles) was used as control. MB001(DE3) (red, squares) and MB001(DE3)::P_{T7}*PduABJknt* (light green, squares) produced AdhB/Pdc, D18-GGSG-AdhB/P18Pdc or D18-GGSG-AdhB/P18Pdc and showed very similar growth performance. Induction of PduABJknt production (50 µM, dark green) resulted in identically declined growth in all strains.

Supplementary references

[1] Parsons, J. B., Frank, S., Bhella, D., Liang, M., Prentice, M. B., Mulvihill, D. P., and Warren, M. J. (2010) Synthesis of empty bacterial microcompartments, directed organelle protein incorporation, and evidence of filament-associated organelle movement, *Mol. Cell 38*, 305-315.

[2] Parsons, J. B., Dinesh, S. D., Deery, E., Leech, H. K., Brindley, A. A., Heldt, D., Frank, S., Smales, C. M., Lunsdorf, H., Rambach, A., Gass, M. H., Bleloch, A., McClean, K. J., Munro, A. W., Rigby, S. E., Warren, M. J., and Prentice, M. B. (2008) Biochemical and structural insights into bacterial organelle form and biogenesis, *J. Biol. Chem. 283*, 14366-14375.

[3] Lawrence, A. D., Frank, S., Newnham, S., Lee, M. J., Brown, I. R., Xue, W. F., Rowe, M. L., Mulvihill, D. P., Prentice, M. B., Howard, M. J., and Warren, M. J. (2014) Solution structure of a bacterial microcompartment targeting peptide and its application in the construction of an ethanol bioreactor, *ACS Synth. Biol. 3*, 454-465.

[4] Hanahan, D. (1983) Studies on transformation of *Escherichia coli* with plasmids, *J. Mol. Biol. 166*, 557-580.

[5] Baumgart, M., Unthan, S., Rückert, C., Sivalingam, J., Grünberger, A., Kalinowski, J., Bott, M., Noack, S., and Frunzke, J. (2013) Construction of a prophage-free variant of *Corynebacterium glutamicum* ATCC 13032 for use as a platform strain for basic research and industrial biotechnology, *Appl Environ Microbiol 79*, 6006-6015.

[6] Kortmann, M., Kuhl, V., Klaffl, S., and Bott, M. (2015) A chromosomally encoded T7 RNA polymerase-dependent gene expression system for *Corynebacterium glutamicum*: construction and comparative evaluation at the single-cell level, *Microb Biotechnol 8*, 253-265.

[7] Frunzke, J., Engels, V., Hasenbein, S., Gätgens, C., and Bott, M. (2008) Co-ordinated regulation of gluconate catabolism and glucose uptake in *Corynebacterium glutamicum* by two functionally equivalent transcriptional regulators, GntR1 and GntR2, *Molecular Microbiology 67*, 305-322.

[8] Kirchner, O., and Tauch, A. (2003) Tools for genetic engineering in the amino acid-producing bacterium *Corynebacterium glutamicum*, *Journal of Biotechnology 104*, 287-299.

[9] Peters-Wendisch, P. G., Schiel, B., Wendisch, V. F., Katsoulidis, E., Mockel, B., Sahm, H., and Eikmanns, B. J. (2001) Pyruvate carboxylase is a major bottleneck for glutamate and lysine production by *Corynebacterium glutamicum*, *J Mol Microbiol Biotechnol 3*, 295-300.

[10] Parsons, J. B., Frank, S., Bhella, D., Liang, M., Prentice, M. B., Mulvihill, D. P., and Warren, M. J. (2010) Synthesis of empty bacterial microcompartments, directed organelle protein incorporation, and evidence of filament-associated organelle movement, *Mol Cell 38*, 305-315.

[11] Hentschel, E., Will, C., Mustafi, N., Burkovski, A., Rehm, N., and Frunzke, J. (2013) Destabilized eYFP variants for dynamic gene expression studies in *Corynebacterium glutamicum*, *Microb Biotechnol 6*, 196-201.

[12] Schäfer, A., Tauch, A., Jäger, W., Kalinowski, J., Thierbach, G., and Pühler, A. (1994) Small mobilizable multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium glutamicum, *Gene 145*, 69-73.

[13] Aussignargues, C., Paasch, B. C., Gonzalez-Esquer, R., Erbilgin, O., and Kerfeld, C. A. (2015) Bacterial microcompartment assembly: The key role of encapsulation peptides, *Commun. Integr. Biol. 8*, e1039755.

[14] Pallerla, S. R., Knebel, S., Polen, T., Klauth, P., Hollender, J., Wendisch, V. F., and Schoberth, S. M. (2005) Formation of volutin granules in *Corynebacterium glutamicum*, *FEMS Microbiol Lett 243*, 133-140.