Supporting Information S1

For the purification of the heterologously expressed microcompartments, we basically followed the method of Sinha *et al.* [1] and added an affinity purification step to provide essentially pure preparations of reprogrammed BMCs for our enzymatic *in vitro* assays. The purification protocol (Fig. S1) was established for the βGal enzyme and was later on successfully applied for the purification of Est5 and GldA.

Expression of the tagged βGal in absence of BMCs showed the highest specific activity in crude extracts of *E. coli*. Co-expression of BMC-proteins lowered the specific activity by roughly 30%. The specific activity of untagged βGal co-expressed with BMCs was the lowest. Expression of large molecular complexes like BMCs will cause some metabolic burden that might reduce the synthesis of βGal, thus accounting for lowered specific activities. Moreover, presence of the encapsulation peptide had a strong stimulating effect on the specific activity of βGal.

The second centrifugation step (20000x*g*) effectively removed all soluble (*i.e.* non-incorporated) βGal activity from the BMC-preparations. The resuspended pellet (containing the BMCs) showed slightly increased specific activity for the tagged βGal. In contrast, activity of tagged βGal expressed in absence of BMCs was exclusively detected in the supernatant. The pellet fraction of untagged βGal co-expressed with BMCs retained only 14% of the specific activity determined for the crude extract. Thus, coincidental incorporation of untagged enzymes during assembly of the shell accounts for only minor activities of the reprogrammed BMCs.

The specific activity of βGal after the centrifugation (12000x*g*, “cleared pellet”; Fig. S1) and Ni-bead treatment stayed roughly constant, and SDS-PAGE analysis revealed only a minor increase in purification efficiency (Fig. S1). Thus, we demonstrate that BMC preparations after the second centrifugation step were already essentially depleted from contaminating βGal species. Centrifugation at 20000x*g* therefore separates most of the soluble (*i.e.*

unbound) βGal from BMC-associated species. We did the same analyses with the esterase Est5 associated to BMCs and the dehydrogenase GldA, and obtained similar results. Together with our analysis of the imidazole eluates from Ni-beads after three successive rounds of affinity purification, we conclude that the determined activities in our *in vitro* assays are exclusively based on enzymes encapsulated by or associated with BMCs, and not by unbound or poorly sheltered enzymes.

Supporting Figure S1

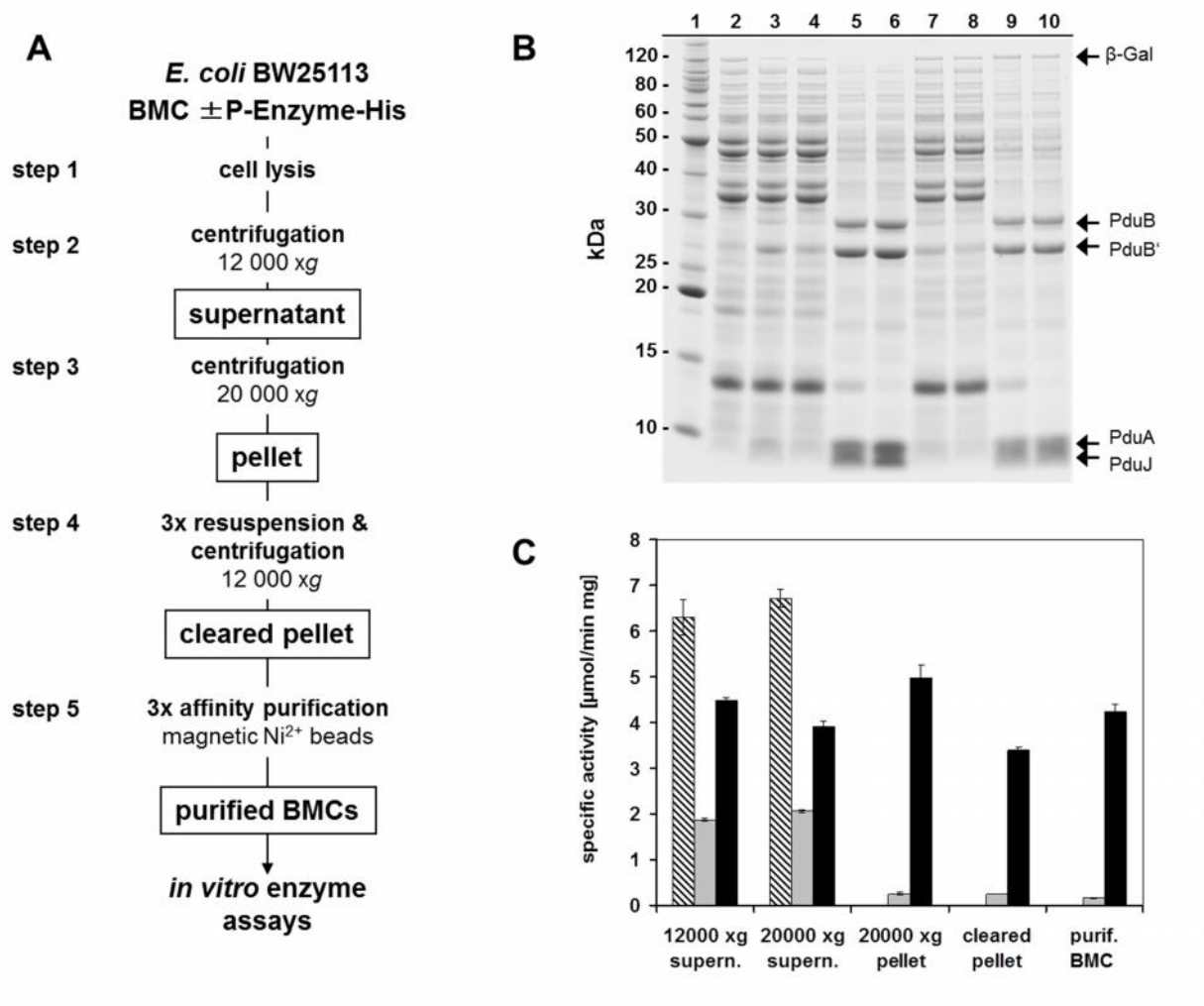

Figure S1. Preparation of reprogrammed BMCs for *in vitro* testing. (A) Flowchart of the protocol applied to purify bacterial microcompartments (BMC). BMC-containing fractions are boxed. Low-speed centrifugation in step 4 removes unspecific particles from the resuspended pellet of step 3; this suspension is termed “cleared pellet”. **(B)** SDS-PAGE

analysis of the purification of BMCs reprogrammed with β-galactosidase (β–Gal). β–Gal was expressed as a His-tag fusion protein (C-terminus) with (P-βGal) or without (βGal) the N-terminal targeting peptide (P) of the PduP-enyzme, and in absence or presence of BMC-genes. Lane 1, molecular mass markers; lane 2, expression of P-βGal in absence of BMC-genes, 12000x*g* supernatant; **lanes 3-6**, co-expression of Pdu-BMCs and βGal; lane 3, 12000x*g* supernatant; lane 4, 20000x*g* supernatant; lane 5, cleared pellet; lane 6, supernatant after immunoprecipitation using magnetic Ni^{2+} beads; **lanes 7-10** co-expression of pdu-BMCs and P-βGal; lane 7, 12000x*g* supernatant; lane 8, 20000x*g* supernatant; lane 9, cleared pellet; lane 10, Ni^{2+} beads supernatant. **(C)** βGal or P-βGal was co-expressed with Pdu-BMCs, respectively. Reprogrammed BMCs were purified as shown in (A). βGal activities of cellular extracts (lysate) or purified BMCs were monitored by an oNPG assay. βGal expressed in absence of BMCs yielded no pellet upon centrifugation at 20000x*g*. Hatched bars, P-βGal, no BMCs; grey bars, βGal co-expressed with BMCs; dark bars, P-βGal co-expressed with BMCs.

Supporting Figure S2

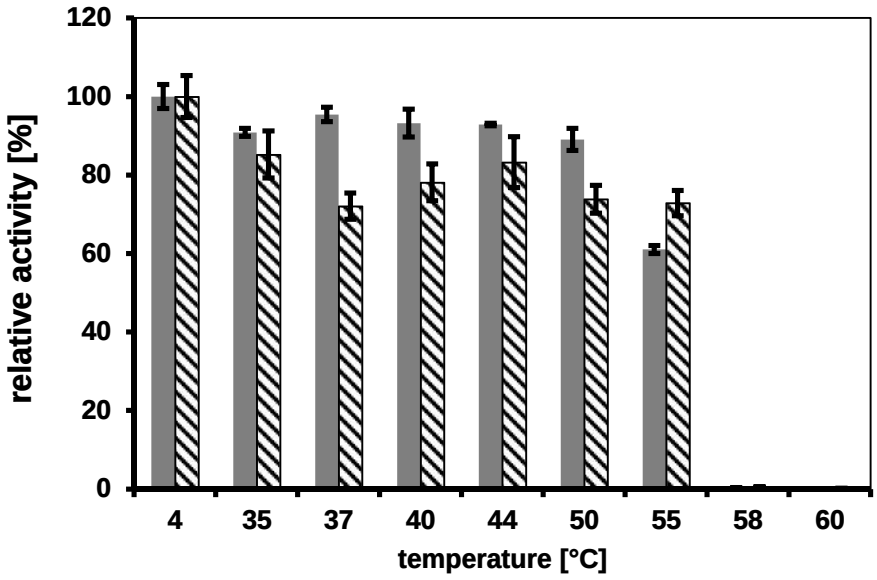

Figure S2 BMCs do not protect -galactosidase from heat denaturation. His-tagged β-glactosidase (βGal) of *E. coli* W3110 was fused to the PduP-encapsulation peptide and

expressed in *E. coli* in presence or absence of BMCs. After affinity purification of the free enzyme or the βGal containing BMCs, both protein solutions were incubated for 15 min at the indicated temperature. Thereafter, β-galactosidase activity was determined by the standard oNPG-assay. Activities were normalized to those determined for the incubation at 4°C. Grey bars, βGal associated with BMCs; hatched bars, βGal without BMCs.

Supporting Figure S3

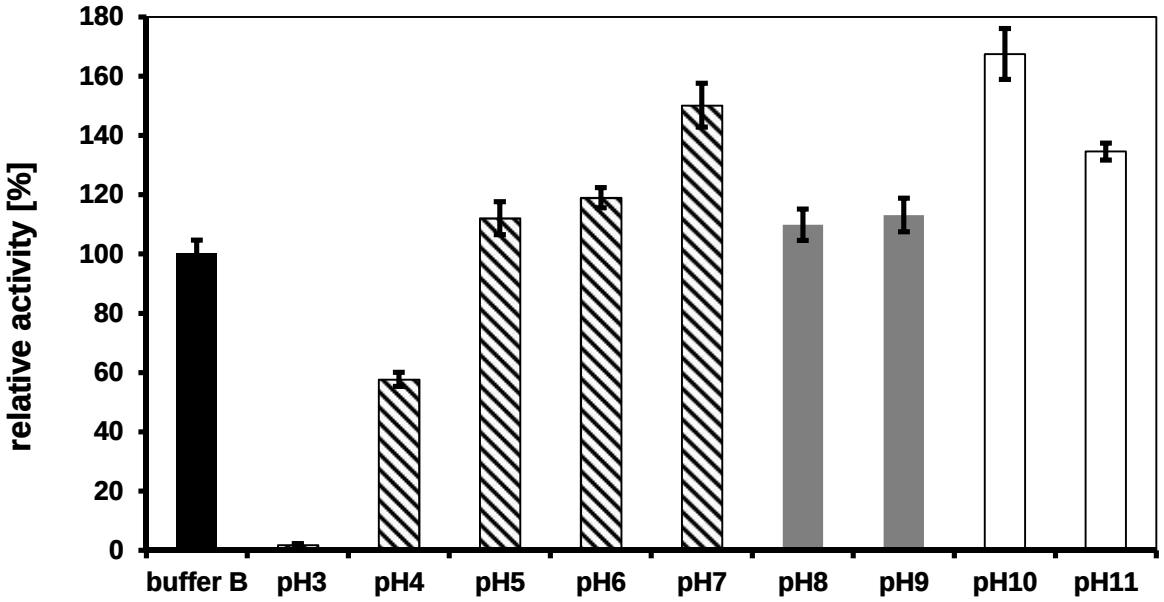

Figure S3 Activity of -galactosidase fused to the Pdu-P encapsulation peptide but not associated with microcompartments after incuabtion in buffers with different pH. β-glactosidase (βGal) of *E. coli* W3110 was fused to the PduP-encapsulation peptide and expressed in *E. coli* in absence of BMCs. After affinity purification of the free enzyme, protein solutions were incubated for 60 min at 4°C in different buffer solutions indicated by the differentially labelled bars. Thereafter, protein solutions were diluted threefold in 100 mM potassium phosphate buffer, pH 7.0, and 50 µl were subsequently used in the standard oNPG assay for the determination of the specific activity. Activities were normalized to the activity of βGal incubated in buffer B (50 mM Tris/HCl, 50 mM KCl, 12.5 mM $MgCl_2$, pH 8.0). The pH of the buffers was adjusted with NaOH or HCl to the respective value. Black bar,

buffer B; hatched bars, 50 mM citrate buffer; grey bars, 50 mM Tris-buffer; white bars, 100 mM CAPS-buffer.

Supporting Table S4

Table S4 Specific activity of -galactosidase or esterase Est5 with or without the PduP-encapsulation peptide (EP) determined by standard assays. One unit equals 1 µmol/min mg protein.

	specific activity [U/mg]		rel. activity [%]
βGal		stdev.	
w/o EP	0.28	0.02	100
PduP-EP	0.83	0.06	293
Est5			
w/o EP	0.12	0.03	100
PduP-EP	0.07	0.01	58

Supporting Table S5

Table S5 Specific activities of the enzymes determined with different substrates.

			specific activity					
				soluble enzyme		BMC-associated		ratio
enzyme	substrate	logP	[unit]	Sup	stdev	BMC	stdev	BMC /Sup
βGal	lactose	**-4,7**	µmol/h mg	**82**	7	**62**	7	**0,76**
	oNPG	**-1,2**	µmol/min mg	**3,9**	0,11	**4,2**	0,17	**1,08**
	MUG	**-0,8**	rfu/min mg	**5003305**	16913	**3230777**	181120	**0,65**
GldA	acetol	**-0,7**	µmol/min mg	**1,4**	0,2	**1,1**	0	**0,79**
	MG	**-0,4**	µmol/min mg	**2,1**	0,2	**1**	0,1	**0,48**
Est5	pNPB	**2,3**	µmol/h mg	**4,3**	0,3	**0,5**	0	**0,12**

βGal, β-galactosidase; GldA, glycerol dehydrogenase; Est5, esterase Est5; oNPG, o-Nitrophenyl-β-galactoside; MUG, 4-Methylumbelliferyl β-D-galactopyranoside; MG, methylgyoxal; pNPB, p-Nitrophenyl butyrate; rfu, relative fluorescence units; Sup, supernatant, i.e. non BMC-associated enzymes; BMC, BMC-associated enzymes.

Supporting Table S6

Table S6 Physical and chemical properties of compounds shown or supposed to cross the shell of the Pdu-BMC.

	role in bioconversion				physical / chemical properties*[1]		
	type	enzyme*[2]	evidence*[3]	formula	MW	logP	source
substrate / intermediate / product of native Pdu-BMC							
Propionaldehyde	i	PduCDE, -P	exp.	C_3H_6O	58,1	0,6	[2]
Propanol	p	PduQ	hyp.	C_3H_8O	60,1	0,3	[3]
Propionic acid*[2]	p	PduW	hyp.	$C_3H_6O_2$	74,1	0,3	[3]
1,2 Propanediol	s	PduCDE	exp.	$C_3H_8O_2$	76,1	-0,9	[4]
Glycerol	s	PduCDE	exp.	$C_3H_8O_3$	92,1	-1,8	[5]
ATP	s	PduO	hyp.	$C_{10}H_{16}N_5O_{13}P_3$	507,2	-5,7	[4]
NAD	s/p	PduP, -Q	exp.	$C_{21}H_{26}N_7O_{14}P_2^-$	662,1	-6,1	[6]
NADH	s/p	PduP, -Q, -S	exp.	$C_{21}H_{29}N_7O_{14}P_2$	665,4	-5,7	[6]
HS-CoA / coenzyme A*[4]	s	PduP	hyp.	$C_{23}H_{38}N_7O_{17}P_3S$	809,6	-5,6	[3]
Propionyl-CoA*[4]	p	PduP	hyp.	$C_{24}H_{40}N_7O_{17}P_3S$	823,6	-5,3	[3]
Cob(I)alamin*[4]	c	PduCDE	hyp.	$C_{62}H_{88}CoN_{13}O_{14}P^{-2}$	1329,3	n.d.	[3]
substrate / detected product of reprogrammed Pdu-BMCs							
Ethanol	p	Adh	exp.	C_2H_6O	46,1	-0,1	[7]
Methylglyoxal (MG)	s	GldA	exp.	$C_3H_4O_2$	72,1	-0,4	this study
Acetol	s	GldA	exp.	$C_3H_6O_2$	74,1	-0,7	this study
Pyruvate	s	Pdc	exp.	$C_3H_3O_3^-$	87,0	-0,6	[7]

o-Nitrophenol	p	βGal	exp.	$C_6H_5NO_3$	139,1	1,8	this study
p-Nitrophenol	p	Est5	exp.	$C_6H_5NO_3$	139,1	1,9	this study
β-D-Galactose	p	βGal	exp.	$C_6H_{12}O_6$	180,1	-2,6	this study
p-Nitrophenyl butyrate (pNPB)	s	Est5	exp.	$C_{10}H_{11}NO_4$	209,2	2,3	this study
o-Nitrophenyl-β-galactoside (oNPG)	s	βGal	exp.	$C_{12}H_{15}NO_8$	301,2	-1,2	this study
4-Methylumbelliferyl β-D-galactopyranoside (MUG)	s	βGal	exp.	$C_{16}H_{18}O_8$	338,3	-0,8	this study
β-D-Lactose	s	βGal	exp.	$C_{12}H_{22}O_{11}$	342,3	-4,7	this study
NADH	s	GldA	exp.	$C_{21}H_{29}N_7O_{14}P_2$	665,4	-5,7	this study

I, intermediate product in respective pathway; s, substrate; p, product; c, cofactor; exp, experimental evidence; hyp, hypothetical evidence; MW, moecular weight; logP, partition coefficient octanol-water

Supporting Figure S7

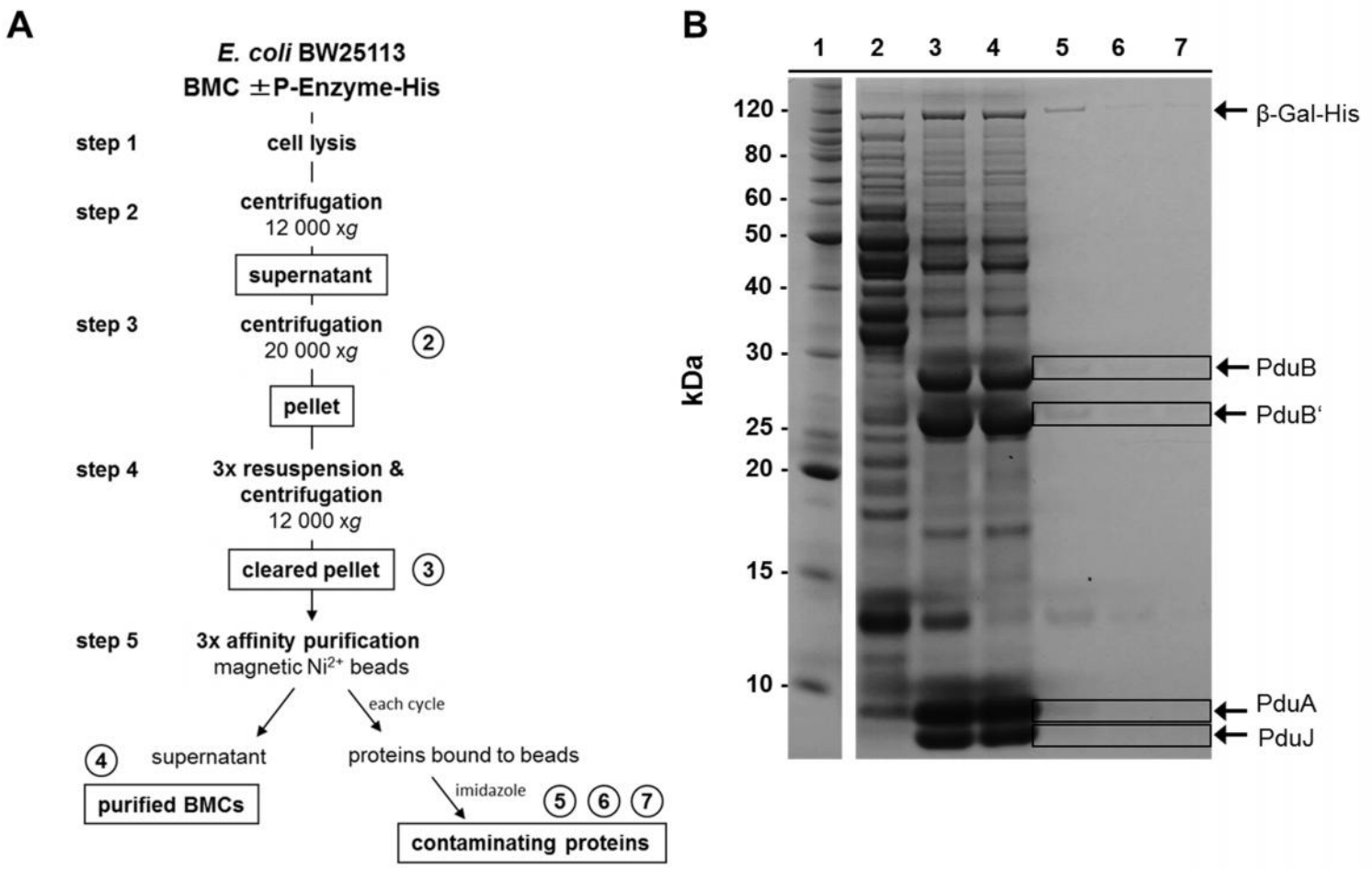

Figure S7. Analysis of the amount of contaminating proteins that are depleted during the BMC-purification protocol. (A) Flowchart of the protocol applied to analyse the amount of proteins that either kept associated or did not associate with BMCs during the individual BMC-purification steps. Proteins that could be separated from the BMCs during purification are collectively termed "contaminating proteins". Numbers in circles indicate the samples applied to SDS-PAGE analysis shown in (B); numbering matches lane numbering in (B). **(B)** SDS-PAGE of samples containing reprogrammed BMCs or contaminating proteins. His-tagged β-galactosidase (β-Gal-His) was targeted to the BMCs via the PduP-encapsulation peptide. Boxes mark hardly detectable, faint protein bands that correspond to the major BMC-shell proteins. Lane 1, molecular mass markers; lane 2, supernatant of the 20000x*g* step, essentially depleted of BMCs; lane 3, 12000x*g* pellet, containing BMCs; lane 4, supernatant after three successive Ni^{2+}-bead treatments, containing purified BMCs; lane 5 - 7, proteins bound to the Ni^{2+}-beads that were used to capture His-tag accessible proteins from the BMC-containing solution; captured proteins were eluted from the beads with imidazole.

Supporting Information S8

Sequence of the PduP-encapsulation peptide (19 AA, bold print) fused to the N-terminus of the payload proteins via a glycine-serine-linker peptide:

Sequence, N-terminus - C-terminus:
MNTSELETLIRTILSEQLTGGGGS-*Mnnnnn* (protein of interest)
Sequence, 5´- 3´ (PduP-peptide fused to GGGS-linker peptide):
ATGAATACTTCTGAACTCGAAACCCTGATTCGCACCATTCTTAGCGAGCAATTAACCGGT
GGAGGCGGGAGT

Table S8 List of primer pairs used for amplification and USER-cloning of shell-protein genes into vector pCC1

Pirmer No.	Template for amplification	Primer sequence 5´- 3´	Reverse primer No	PCR-product
1	pduA	GGGTTTAAUAAGAAGGAGATATACATATGCAACAAGAAGCACTAGG	2	pduAB
2	pduB	AGGATTTCGTAUTCAGATGTAGGACGGACGATCG	1	pduAB
3	pduJ	ATACGAAATCCUAAGAAGGAGATATACATATGAATAACGCACTGGGACTGG	4	pduJK
4	pduK	AGGCCCGCGAUTTACGCTTCACCTCGCTTGC	3	pduJK
5	pduM	ATCGCGGGCCUAAGAAGGAGATATACATATGAACGGCGAAACCCTGC	6	pduMN
6	pduN	ATTCGCTAACUTTAACACGAAAGCGTATCTAC	5	pduMN
7	pduT	AGTTAGCGAAUAAGAAGGAGATATACATATGTCTCAGGCTATAGG	8	pduTU
8	pduU	GGTCTTAAUTTACGTCCGGGTGAT	7	pduTU

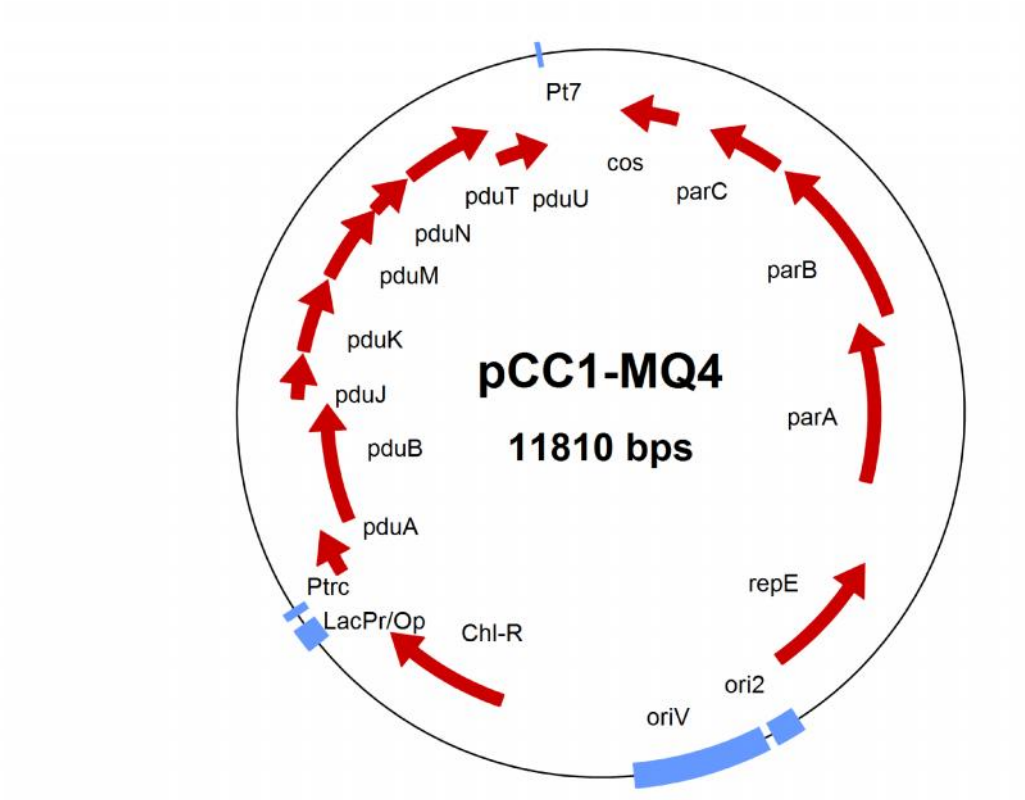

Figure S8-1: Map of plasmid pCC1-MQ4 encoding the proteins for minimal-shell BMCs. pduA -U, genes encoding the BMC minimal shell proteins; Ptrc, promoter driving the BMC-gene expression; LacPr/Op, binding site for LacI repressor (not encoded on plasmid); Chl-R, chloramphenicol acetyltranserfase; oriV, high copy origin of replication (inactive); ori2, repE, parA,-B,-C, elements for single-copy replication; cos, cohesive-site; Pt7, T7-promoter.

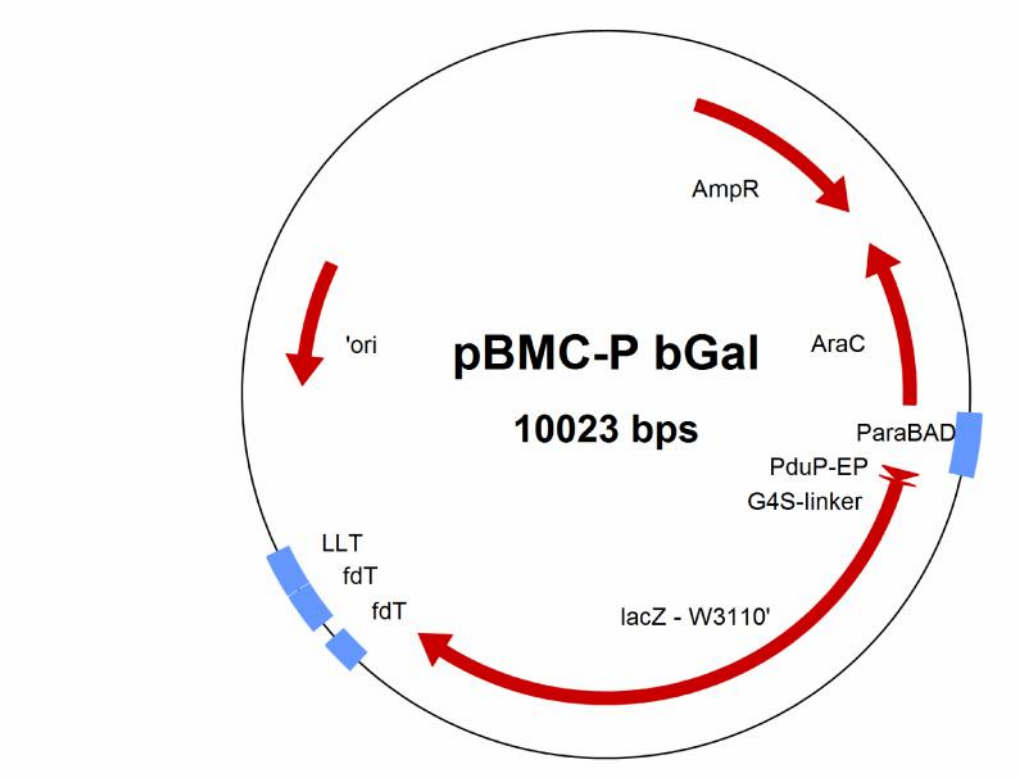

Figure S8-2: Map of plasmid pBMC-P encoding -Galactosidase of *E. coli* W3110 fused to the PduP-encapsulation peptide. The encapsulation peptide is fused to the N-terminus of βGal via a GGGGS-peptide linker (G4S) and expression of βGal is under control of the L-arabinose inducible ParaBAD-AraC system. ori, origin of replication (high copy number), LLT, fdT, termination sequences; AmpR, β-lactamase, ampicillin resistance marker.

Supporting Information References

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