Supplementary Information for:

Effect of bio-engineering on size, shape, composition and rigidity of bacterial microcompartments.

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TEM of cells forming Pdu metabolosomes and empty microcompartments

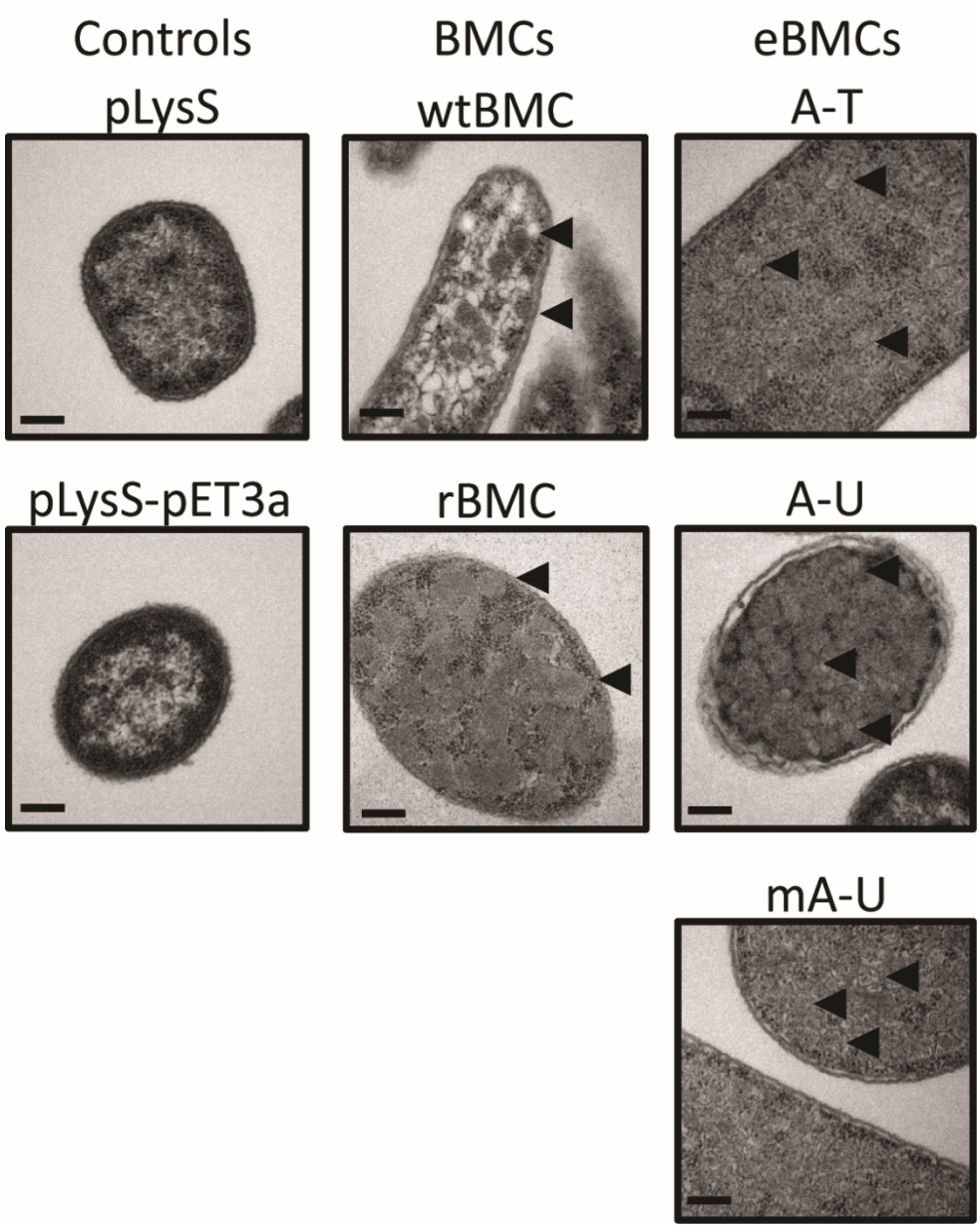

Figure 1. Electron microscopy images of strains transformed with control vectors (pLysS, pLysS-pET3a), wild-type *C. freundii,* recombinant operon (rBMC) and empty microcompartment constructs (eBMCs: A-T, A-U and A-T) [17, 19, 35, 38]. Scale bars correspond to 200 nm.

Purification of empty Pdu microcompartments (eBMC) and Pdu metabolosomes (BMC)

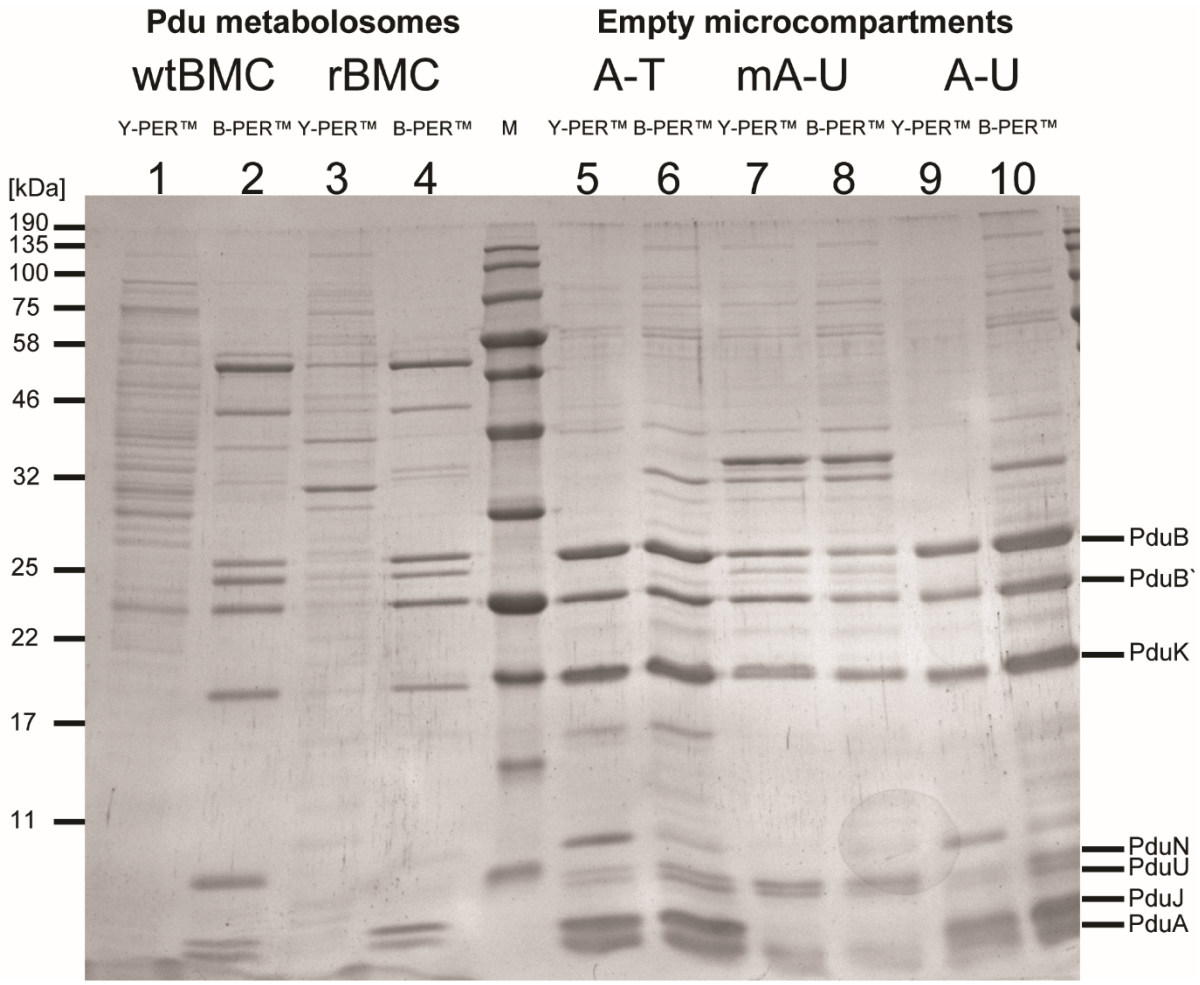

Figure 2. Summary of microcompartment purification. A 15 % polyacrylamide gel is show. 10 μl samples were loaded in each lane, (M) represents the marker lane. The gels show that empty microcompartment constructs (A-T, mA-U and A-U) can be purified (lanes 5-10) using previously published methods using bacterial protein extraction reagent (B-PER™) or yeast extraction reagent (Y-PER™). Metabolosomes (rBMC and wild type BMC) did not purify using the Y-PER™ method (lanes 1 and 3) but purified with B-PER™ (lanes 2 and 4). The expected molecular mass of the shell proteins are as follows: PduB = 28 KDa, PduB' = 23 KDa, PduK = 16 KDa, PduN = 9.2 KDa, PduU = 12.5 KDa, PduJ = 9.0 KDa, PduA = 9.5 KDa.

MALDI MS-MS

The following figure shows 2D-PAGE gels with the corresponding protein spots identified by tandem mass spectrometry consistent with previously reported proteomic data [16, 38].

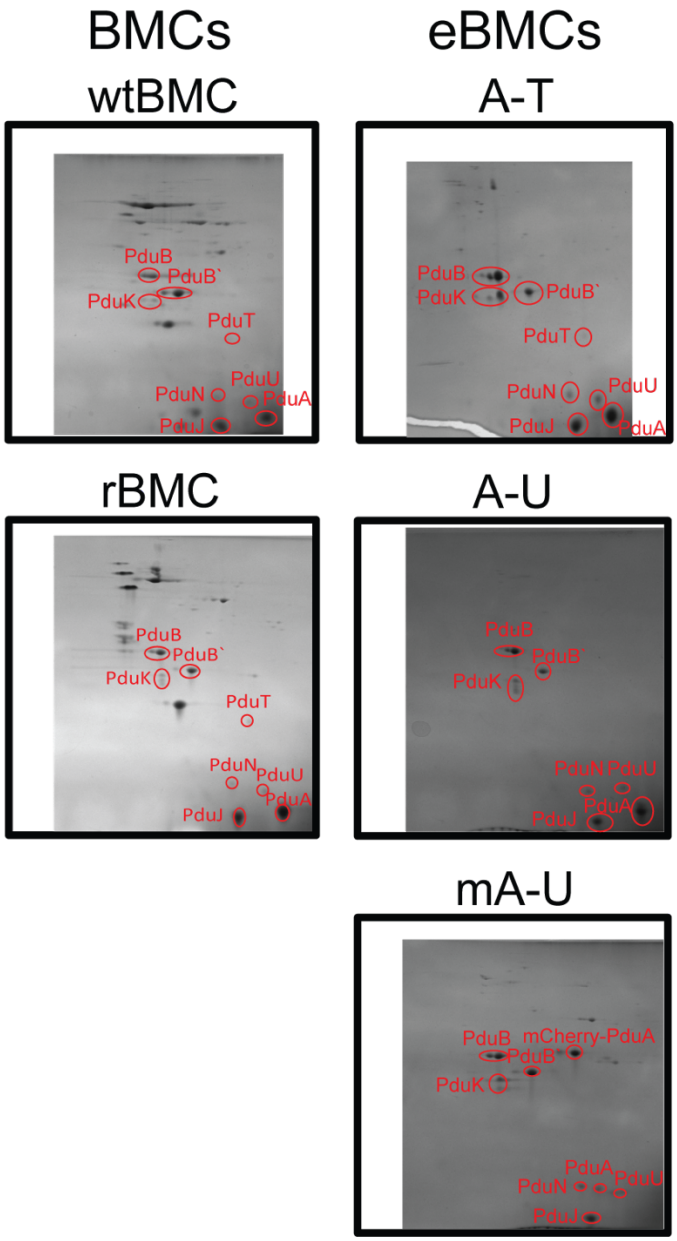

Figure 3. 2D-PAGE gels of purified Pdu microcompartments. Shell protein spots identified by MALDI TOF and TOF-TOF are shown in red.

Single particle analysis (diameter/area/height):

Table 1. The following table comprise the statistical analysis for diameter [nm] studies. Average, minimum, maximum, median and standard deviation for Pdu metabolosomes (wtBMC, rBMC) and empty microcompartments (A-T, A-U and mA-U) and. TEM and AFM values are given each.

	Pdu metabolosomes				**Empty microcompartments**					
Diameter [nm]	**wtBMC**		**rBMC**		**A-T**		**A-U**		**mA-U**	
	TEM	**AFM**	**TEM**	**AFM**	**TEM**	**AFM**	**TEM**	**AFM**	**TEM**	**AFM**
Average	127	131	122	133	77	59	65	66	74	56
Minimum	94	94	72	80	17	36	50	49	38	36
Maximum	162	175	201	202	126	80	84	96	186	80
Median	127	129	117	129	75	62	65	64	68	50
Standard deviation	13.2	15.4	26.4	23.6	18.5	7.9	7.4	8.4	23.0	8.0

Table 2. The following table comprise the statistical analysis for area [μm^2] studies. Mean, minimum, maximum, median and standard deviation are given for Pdu metabolosomes (wtBMC and rBMC) and empty microcompartments (A-T, A-U and mA-U). TEM and AFM values are given each.

Area [μm^2]	Pdu metabolosomes				Empty microcompartments					
	wtBMC		rBMC		A-T		A-U		mA-U	
	EM	AFM	EM	AFM	EM	AFM	EM	AFM	EM	AFM
Average	0.0128	0.0137	0.0122	0.0143	0.0049	0.0028	0.0034	0.0034	0.0047	0.0025
Minimum	0.0070	0.0070	0.0041	0.0050	0.0002	0.0010	0.0019	0.0019	0.0011	0.0010
Maximum	0.0205	0.0240	0.0316	0.0320	0.0124	0.0050	0.0056	0.0073	0.0272	0.0050
Median	0.0126	0.0130	0.0108	0.0130	0.0044	0.0030	0.0033	0.0032	0.0036	0.0020
Standard Deviation	0.0026	0.0032	0.0053	0.0053	0.0024	0.0007	0.0008	0.0009	0.0033	0.0007

Table 3. The following table comprise the statistical analysis particle height [nm] studies. Average, minimum, maximum, median and standard deviation are given for Pdu metabolosomes (wtBMC and rBMC) and empty microcompartments (A-T, A-U and mA-U). AFM values are given each.

	Pdu metabolosomes		**Empty microcompartments**		
	wtBMC	**rBMC**	**A-T**	**A-U**	**mA-U**
Height [nm]	**AFM**	**AFM**	**AFM**	**AFM**	**AFM**
Average	49.99	50.15	18.74	18.42	18.46
Minimum	36.00	36.00	12.00	13.00	13.50
Maximum	78.00	71.00	30.00	24.50	31.50
Median	50.00	49.00	18.00	19.00	18.30
Standard Deviation	8.10	8.36	3.31	2.41	3.16

Shell protein composition in empty microcompartments and Pdu metabolosomes

Table 4. Shell protein composition of studied Pdu microcompartment types (wtBMC, rBMC, A-T, A-U, mA-U). Mean values of three independent repeats (N=3) and standard error of mean are given.

		Major shell protein components				**Minor shell protein components**			
		PduA	**PduB**	**PduB`**	**PduJ**	**PduK**	**PduN**	**PduT**	**PduU**
Pdu metabolosomes	**wtBMC**	19.4 ± 0.9	18.4 ± 0.7	30.7 ± 1.5	23.8 ± 1.1	4.2 ± 0.3	2 ± 0.4		1.4 ± 0.2
	rBMC	22.5 ± 0.2	24.9 ± 0.7	27.7 ± 1.7	18.9 ± 0.1	2.4 ± 0.6	2 ± 0.2		1.4 ± 0.4
Empty microcompartments	**A-T**	35.9 ± 3.6	18.3 ± 0.7	12.2 ± 2.7	18.1 ± 1.0	6.6 ± 1.4	3.4 ± 0.3	1.9 ± 0.1	3.6 ± 0.9
	A-U	27.1 ± 4.0	23.6 ± 1.2	16.0 ± 1.4	18.2 ± 1.5	6.0 ± 1.3	5.4 ± 1.4		3.7 ± 1.1
	mA-U	35.3 ± 2.8	18.4 ± 1.7	13.5 ± 1.5	13 ± 1.7	7 ± 0.8	5.2 ± 0.9		7.5 ± 2.4

Strain list

Table 5. Summary of the used strains, bacterial strain, plasmid construct and source/references are noted.

	Bacterial strain	**Plasmid**	**Source/Reference**
Controls	Bl21(DE3)	pLysS	Novagen
	Bl21(DE3)	pLysS, pET3a	Novagen
Pdu metabolosomes	*Citrobacter freundii* ballerup 7851	wtBMC	Provided by the Sanger Institute (Cambridge, United Kingdom)
	Bl21(DE3)-plysS	pET14b-Pdu65 (A-X), rBMC	Parsons *et al.*, 2008
Empty microcompartments	Bl21(DE3)	pLysSA-U(*pduA-B-B`-J-K-N-U*), A-U	Parsons *et al.*, 2010
	Bl21(DE3)	pLysSmCherryA-U(*mcherrypduA-B-J-K-N-U*), mA-U	Parsons *et al.*, 2010
	Bl21(DE3)-plysS	pET3aA-T (*pduA-B-J-K-N-U-T*), A-T	Parsons *et al.*, 2010

Quantitative nano-mechanical mapping

Table 6. Quantitative nano-mechanical mapping at 250 nN did change the observed particle height of individual Pdu microcompartments (wtBMC, rBMC, A-T, A-U and mA-U). Mean values and standard error of mean (of 20 measured particles) are reported of, reduced DMT modulus (E*) [GPa] and deformation.

	Construct	**Reduced DMT modulus (E*) [GPa]**	**Deformation [nm]**
Pdu metabolosomes	wild type	5.4 ± 0.9	2.9 ± 0.1
	rBMC	5.0 ± 0.9	3.0 ± 0.1
Empty microcompartments	A-T	25.3 ± 2.2	2.01 ± 0.1
	A-U	22.8 ± 2.3	1.85 ± 0.1
	mA-U	29.8 ± 3.8	1.68 ± 0.1