

Figure S1

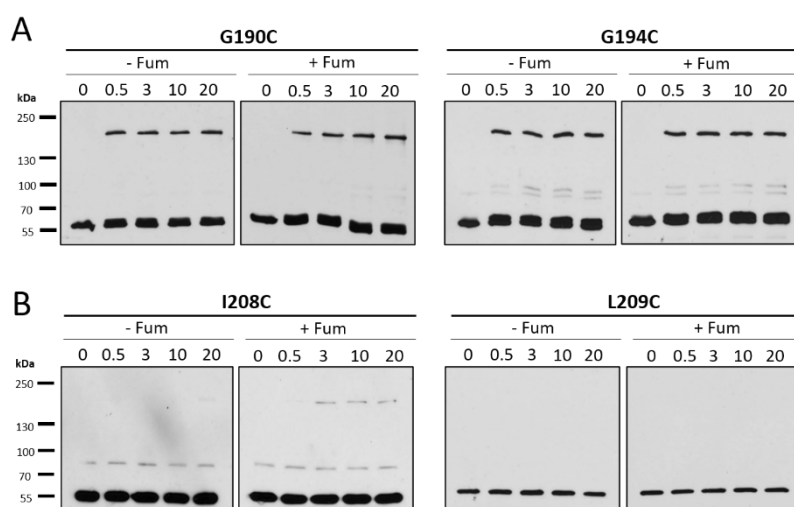


Figure S1. Detection of DcuS TM2 (G190C, G194C) (A) or Linker variants (I208C, L209C) (B), after CL of DctA deficient bacteria (IMW660) with Cu²⁺ phenanthroline. Cross-linking was performed as described in [13]. CL-products were detected by chemiluminescence after non-reducing SDS-PAGE and Western blot. Scans of the X-ray films are shown. The reaction time is shown above the scans (0 to 20 min) and the absence (- Fum) or presence (+ Fum) of fumarate is indicated, as is the respective DcuS variant.

Table S1. Strains and plasmids used.

Strain	Genotype	Reference
BTH101	F ⁻ , <i>cya</i> -99, <i>araD</i> 139, <i>galE</i> 15, <i>galK</i> 16, <i>rpsL</i> 1 (<i>Str^r</i>), <i>hsdR</i> 2, <i>mcrA</i> 1, <i>mcrB</i> 1	[47]
C43(DE3)	F ⁻ , <i>ompT</i> , <i>gal</i> , <i>dcm</i> , <i>lon</i> , <i>hsdSB</i> (<i>rB-mB</i> -), λ (DE3 [<i>lacI lacUV5-T7p07 ind1 sam7 nin5</i>]), spontaneous mutation of BL21(DE3) to overproduce membrane proteins	[48]
JW4086	F ⁻ , Δ (<i>araD-araB</i>)567, Δ <i>lacZ</i> 4787(:: <i>rrnB</i> -3), λ ⁻ , <i>rph</i> -1, Δ (<i>rhaD-rhaB</i>)568, Δ <i>dcuS</i> 751::kan, <i>hsdR</i> 514	[49]
JW3496	F ⁻ , Δ (<i>araD-araB</i>)567, Δ <i>lacZ</i> 4787(:: <i>rrnB</i> -3), λ ⁻ , Δ <i>dctA</i> 783::kan, <i>rph</i> -1, Δ (<i>rhaD-rhaB</i>)568, <i>hsdR</i> 514	[49]
IMW660	C43(DE3) but <i>dcuS</i> ::FRT, <i>dctA</i> ::FRT	This study
pKT25	C-Terminal T25 protein fusion plasmid, pSU40 derivative, KanR	[50]
pKNT25	N-Terminal T25 protein fusion plasmid, pSU40 derivative, KanR	[50]
pUT18	N-Terminal T18 protein fusion plasmid, pUC19 derivative, AmpR	[50]
pUT18C	C-Terminal T18 protein fusion plasmid, pUC19 derivative, AmpR	[50]
pKT25-Zip	^{T25} Zip expression plasmid, pKT25 derivative, KanR	[47]
pMW336	His ₆ -DcuS expression plasmid but DcuS 199S-C471S, pET28-derivative (Kan ^r)	[6]
pMW336	His ₆ -DcuS expression plasmid but DcuS 199S-C471S, pET28-derivative (Kan ^r)	[6]
pMW429	^{T18} DcuS expression plasmid, pUT18C derivative, AmpR	This study
pMW1911	^{T18} PASc expression plasmid, pUT18C derivative, AmpR	This study
pMW1912	^{T18} PASc-Kinase expression plasmid, pUT18C derivative, AmpR	This study
pMW1913	^{T18} L-PASc expression plasmid, pUT18C derivative, AmpR	This study
pMW1914	^{T18} L-PASc-Kinase expression plasmid, pUT18C derivative, AmpR	This study
pMW1126	DctA ₄₀₀₋₄₂₈ -T25 expression plasmid, pKNT25 derivative, KanR	This study
pMW1915	^{T18} L ⁻¹ -PASc expression plasmid, pUT18C derivative, AmpR	This study
pMW1916	^{T18} L ⁻² -PASc expression plasmid, pUT18C derivative, AmpR	This study
pMW1917	^{T18} L ⁻³ -PASc expression plasmid, pUT18C derivative, AmpR	This study
pMW1918	^{T18} L ⁻⁴ -PASc expression plasmid, pUT18C derivative, AmpR	This study
pMW1919	^{T18} L ⁻⁵ -PASc expression plasmid, pUT18C derivative, AmpR	This study

pMW858	^{T25} DctA expression plasmid, pKT25 derivative, KanR	This study
pMW1851	pMW336 but DcuS-I208C (KanR)	[12]
pMW1852	pMW336 but DcuS-L209C (KanR)	[12]
pMW2522	pMW336 but DcuS-G190C (KanR)	[13]
pMW2526	pMW336 but DcuS-G190C (KanR)	[13]
pMW2685	pMW336 but DcuS-R224C (KanR)	This study