Supplementary Information

Uncommon activation of *Syn*DLP, the fusogenic Dynamin-like protein of the cyanobacterium *Synechocystis* sp. PCC 6803

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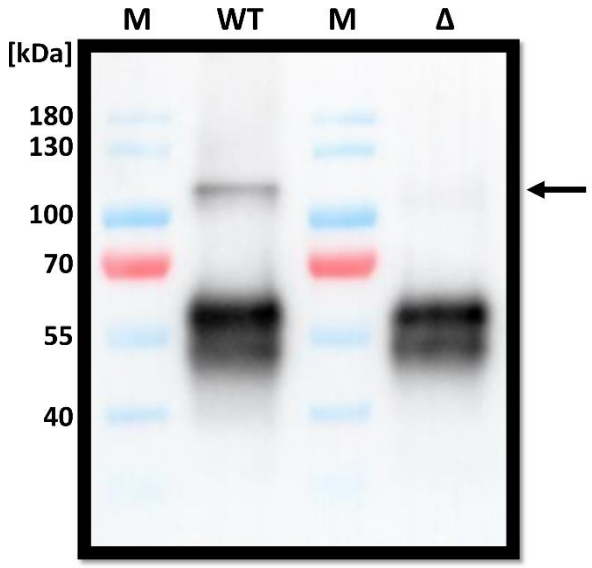
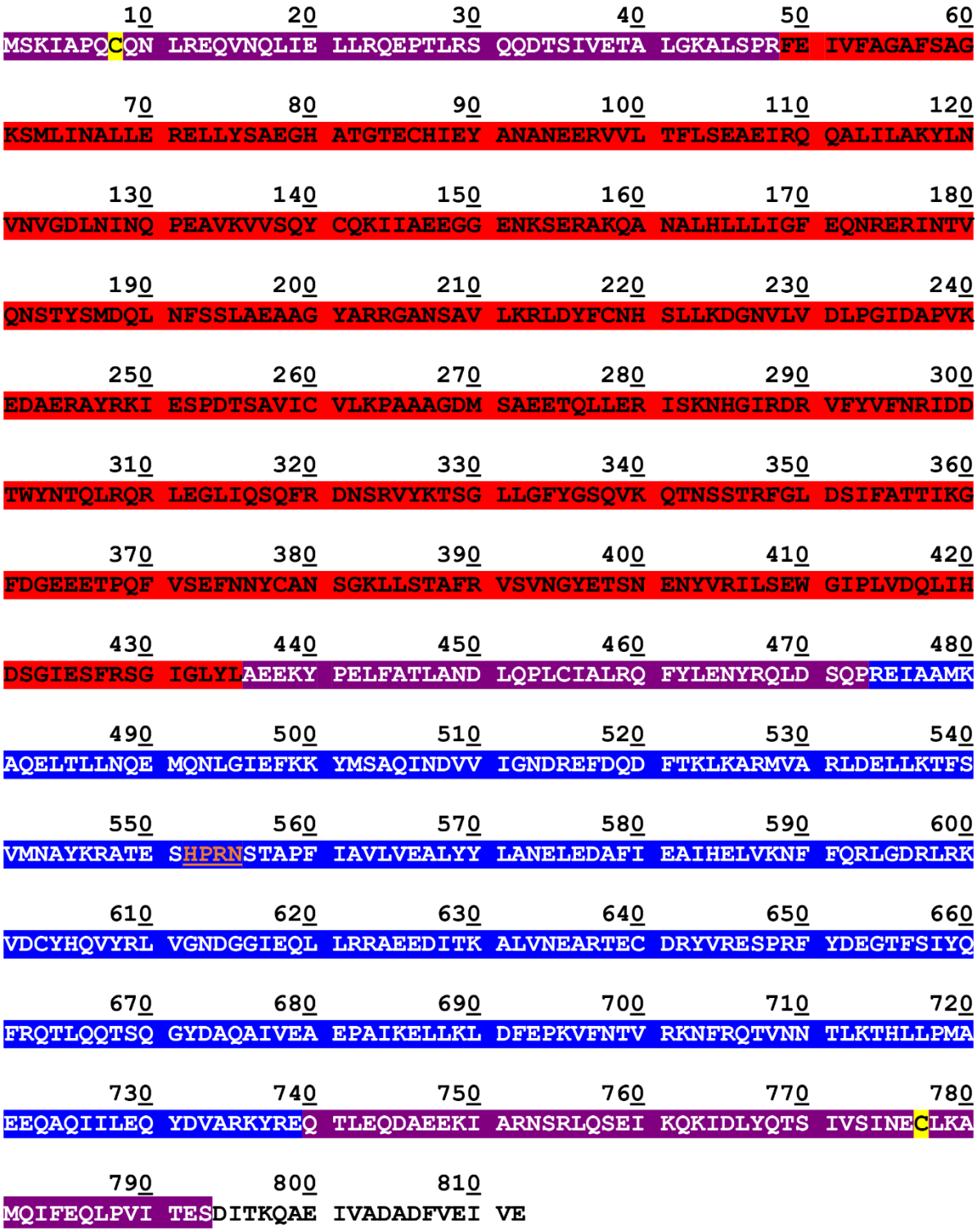
Supplementary Table 1: Primers used for molecular cloning, site-directed mutagenesis and Gibson assembly. F = forward primer, R = reverse primer

Primer	**Used for**	**Sequence (5' → 3')**
F_NsiI_slr0869	Amplification of *slr0869* with NsiI restriction site	GGCCATGCATATGTCCAAGATTGCGCCCCA
R_XhoI_slr0869	Amplification of *slr0869* with XhoI restriction site	GGCCCTCGAGTTCTACTATTTCCACAAAAT
F_TG2329GC	Site-directed mutagenesis C777A	CGAGTATTGTTAGCATTAATGAAGCTTTAA AAGCCATGCAAATTTTTG
R_TG2329GC	Site-directed mutagenesis C777A	CAAAAATTTGCATGGCTTTTAAAGCTTCATT AATGCTAACAATACTCG
F_pET303-*Syn*DLP-HPRN552-555AAAA_insert	Mutation of HPRN552-555AAAA via Gibson assembly, insert PCR	CTTTAAGAAGGAGGTCTAGAATGCATATGT CCAAGATTGCGCCCCAATG
R_pET303-*Syn*DLP-HPRN552-555AAAA_insert	Mutation of HPRN552-555AAAA via Gibson assembly, insert PCR	AAGGAGCTGTGGACGCTGCGGCCGCACTTT CCGTTGCTCGCTTATAGGC
F_pET303-*Syn*DLP-HPRN552-555AAAA_vector	Mutation of HPRN552-555AAAA via Gibson assembly, vector PCR	GCGGCCGCAGCGTCCACAGCTCCTTTTATT GCAGTTTTG
R_pET303-*Syn*DLP-HPRN552-555AAAA_vector	Mutation of HPRN552-555AAAA via Gibson assembly, vector PCR	CATTGGGGCGCAATCTTGGACATATGCATT CTAGACCTCCTTCTTAAAG



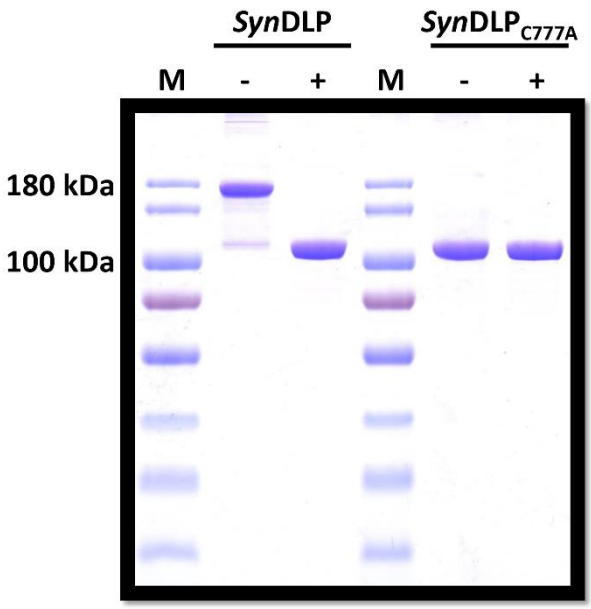
Supplementary Figure 1: *Syn*DLP expression *in vivo*.

100 ml of *Synechocystis* sp. PCC 6803 WT culture and a *Syn*DLP knock-out strain (Δ) were grown in a shaking incubator at 130 rpm, 30°C and illuminated with 30 µE to an OD_{750} = 1.4. After cell disruption, *Syn*DLP was immunoprecipitated using Sepharose-Protein A beads and an antibody raised in a rabbit against parts of *Syn*DLP (α-*Syn*DLP). The immunoprecipitated proteins were separated via SDS-PAGE and *Syn*DLP was visualized by Western Blot analysis using α-*Syn*DLP as primary and α-Rabbit-HRP conjugate as secondary antibody. The height of a *Syn*DLP control band is marked by an arrow. M = marker. The *Syn*DLP knock-out strain was generated by homologous recombination using a plasmid containing flanking regions up- and downstream of the gene coding for *Syn*DLP (*slr0869*) and a kanamycin resistance cassette between the flanking regions. For selection, the strain was grown in BG11 medium containing up to 100 µg/ml kanamycin. Complete segregation was verified by PCR.



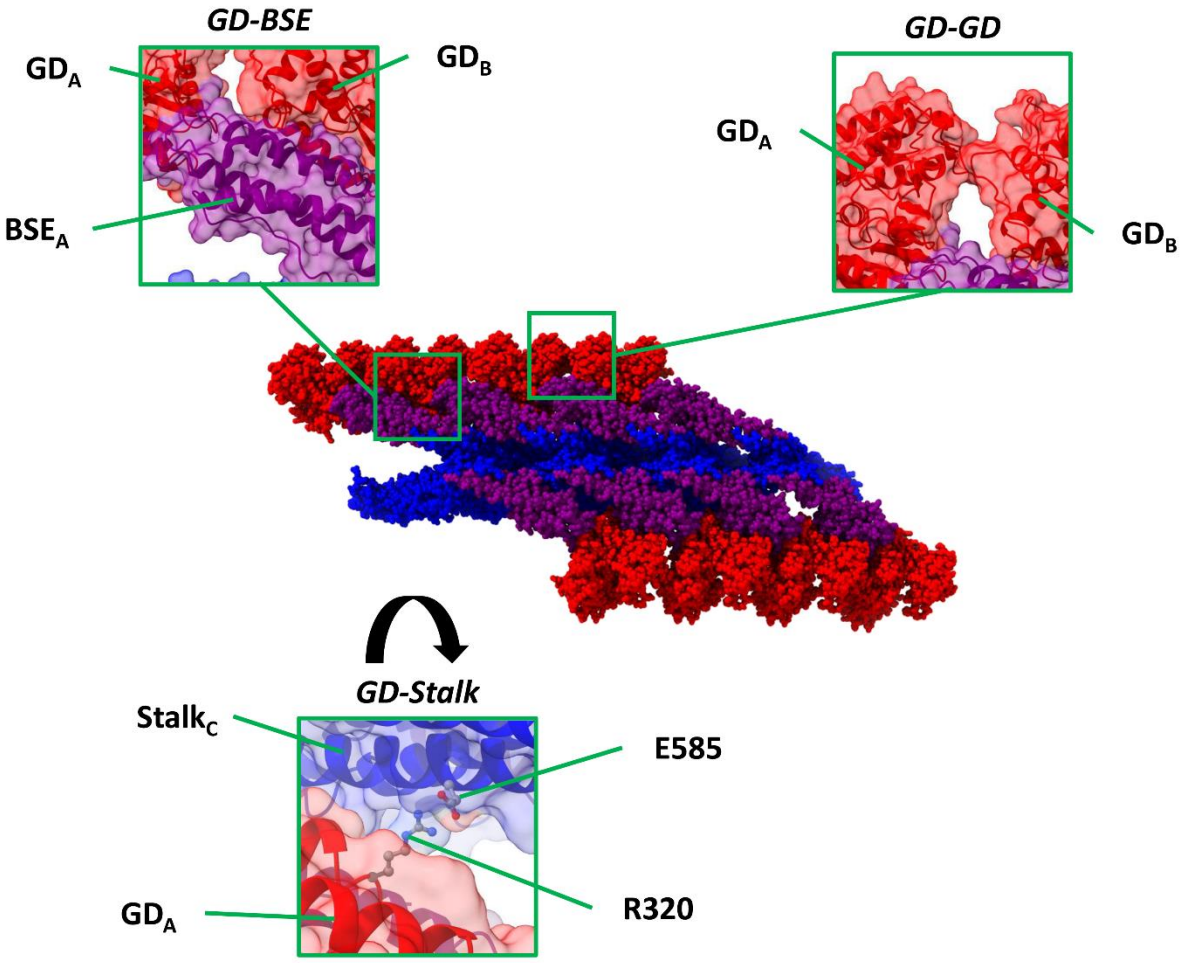
Supplementary Figure 2: Annotated amino acid sequence of *Syn*DLP.

*Syn*DLP domains are highlighted derived from the structure (Fig. 2): BSE1-3 in violet, GD in red, stalk in blue. The four substituted residues in the *Syn*DLP$_{HPRN\text{-}AAAA}$ mutant are typed orange and underlined. The two cysteines forming an intramolecular disulfide bridge are marked in yellow.



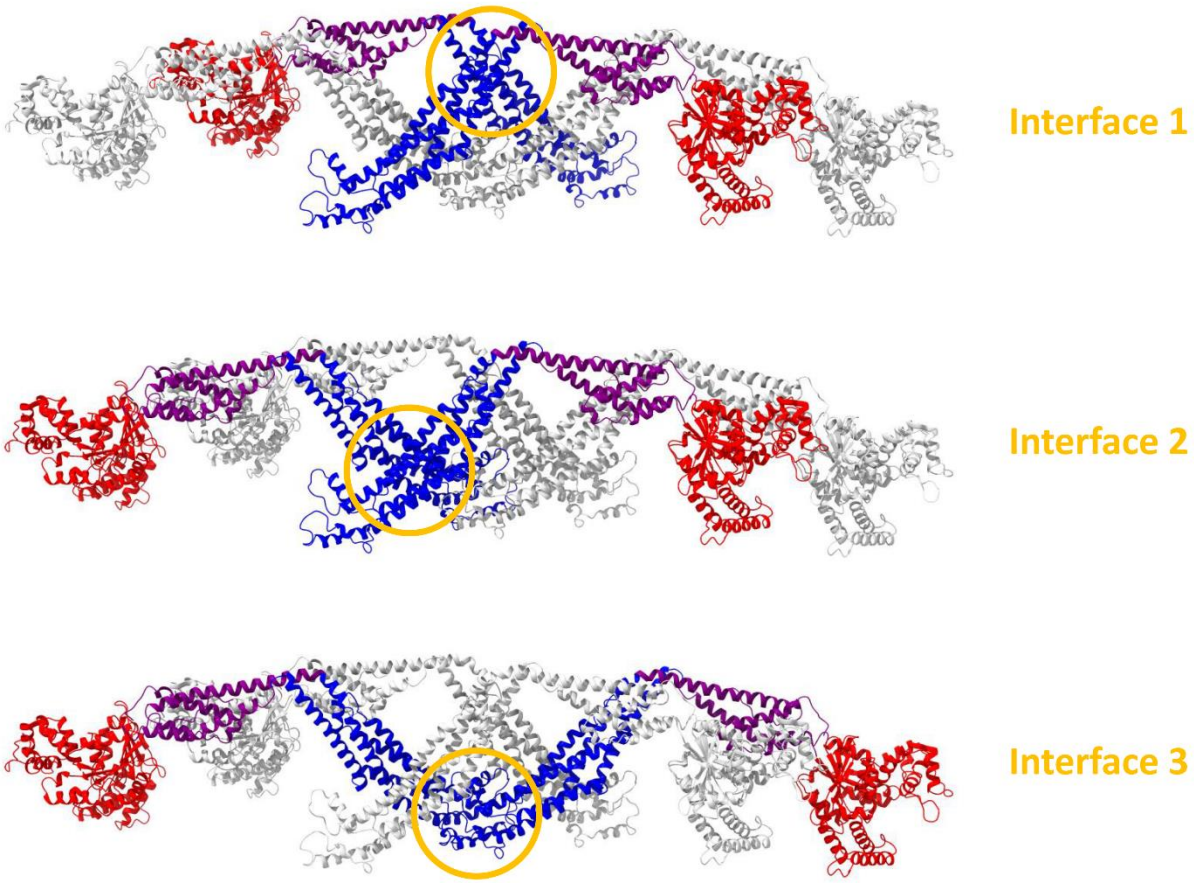
Supplementary Figure 3: The C777A mutation abolishes the formation of the intramolecular disulfide bridge.

Purified *Syn*DLP and *Syn*DLP$_{C777A}$ were analyzed via SDS-PAGE under reducing (100 mM DTT, lane +) and non-reducing (0.1 mM DTT, lane -) conditions. M = marker.



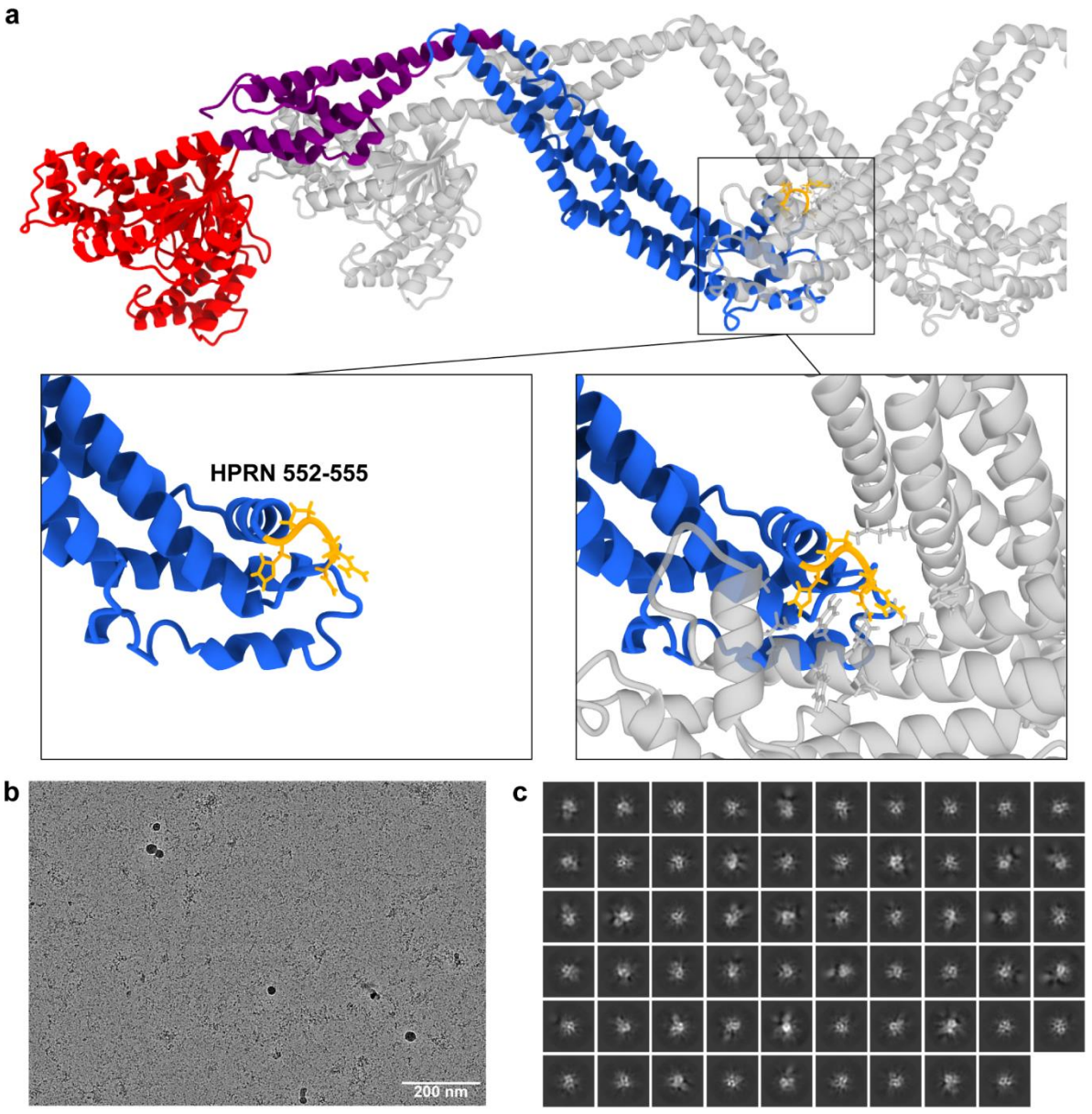
Supplementary Figure 4: Intermolecular domain contacts in the *Syn*DLP oligomer.

Structure of a *Syn*DLP oligomer is shown in the center in surface representation. GD, BSE and stalk are colored red, violet and blue, respectively. The three sections zoom areas of contacts of one GD with another polypeptide chain, either between the GD and the BSE domain (GD-BSE), between two GDs (GD-GD) or between the GD and the stalk domain (GD-stalk). Important single residues mediating GD-stalk contacts (R320 and E585) are shown as balls and sticks and colored by element.



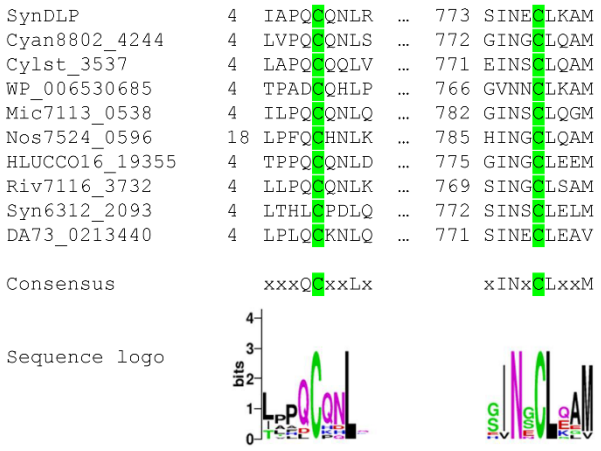
Supplementary Figure 5: Oligomerization interfaces in *Syn*DLP.

An isolated tetramer of the *Syn*DLP oligomer with highlighted subunits containing interfaces 1, 2 and 3 in the stalk region, as they were identified also in other Dynamin superfamily members. Structures shown as ribbon representation. BSE in violet, GD in red, stalk in blue.



Supplementary Figure 6: Mutation of oligomerization interface 3 residues.

(a) An isolated tetramer of the *Syn*DLP oligomer is shown as ribbon representation in grey. The domains are colored in one monomer (GD in red, BSE in purple, stalk in blue). The zoomed sections show four residues (^{552}HPRN^{555}) colored in orange and as sticks that lie on a loop in the oligomerization interface 3 either in one monomer (left) or together with contacting monomers (right). ^{552}HPRN^{555} were substituted to AAAA to impair the assembly of *Syn*DLP oligomers. (b) Cryo-EM micrograph of *Syn*DLP$_{\text{HPRN-AAAA}}$. (c) 2D class averages of *Syn*DLP$_{\text{HPRN-AAAA}}$ particles (250,000 particles with 272 Å box dimension).



Supplementary Figure 7: Disulfide forming cysteine residues are conserved within cyanobacterial KGK clade DLPs.

Sequence alignment of predicted cyanobacterial Dynamin-like GTPases belonging to the KGK clade. The section shows the sequence context of *Syn*DLP C8 and C777 residues together with corresponding sequence regions of other cyanobacterial KGK clade DLPs. Cyan8802_4244 from *Cyanothece* sp. PCC 8802, Cylst_3537 from *Cylindrospermum stagnale* PCC 7417, WP_006530685 from *Gloeocapsa* sp. PCC 73106, Mic7113_0538 from *Microcoleus* sp. PCC 7113, Nos7524_0596 from *Nostoc* sp. PCC 7524, HLUCCO16_19355 from *Phormidium* sp. OSCR, Riv7116_3732 from *Rivularia* sp. PCC 7116, Syn6312_2093 from *Synechococcus* sp. PCC 6312, DA73_0213440 from *Tolypothrix bouteillei* VB521301.