DNA directed binding of photosynthetic light-harvesting proteins**


Supporting Information

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Experimental procedures

**Bacterial growth, strains and media**

*Rps. palustris* was grown anaerobically in C-succinate media in light at 15 μW.m⁻¹ at 30°C as described previously. Rps. palustris cultures for conjugation were grown aerobically in the same media in the dark. After conjugation the Rps. palustris were grown in C-succinate agar plates minus casamino acids (denoted as C-succinate) to select against any remaining *E.coli*. Strain JM109 of *E.coli* was used for all the cloning steps and grown at 37°C in LB media supplemented with antibiotic where indicated. The conjugation into *Rps. palustris* used *E.coli* S-17-1 λ*pir*, a RP4 derivative strain capable of mobilizing plasmid DNA, also containing the *pir* gene to allow for replication of the suicide vector pK18mobsacB.

**DNA cloning and construction of *puhA-zif268***

The genome of *Rps. palustris* CGA009 has been sequenced and is available from GenBank (BX571963.1) and the accession number for the *puhA* gene is NP 946894. Primer pairs were designed for Splice Overlap Extension (SOE) PCR, incorporating an overlap sequence of 18bp to attach two adjacent fragments. A schematic of the *puhA-zif268* construct including position of primers is shown in the Figure S1 and primer sequences listed in Table S1. Primers 1 and 2 have the stop codon of the *puhA* gene removed to allow for continuous transcription to the *zif268*. Phusion DNA polymerase (Finnzymes, Thermo Scientific) was used for all PCR amplifications with the PCR protocol optimized for each of the reactions.

![Schematic illustration of the *puhA-zif268* DNA construct made to attach the *zif268* zinc finger DNA of *zif268* to the *puhA* gene from *Rps. palustris*. The stop codon of the *puhA* gene was removed in primers 1 and 2 and the *zif268* inserted between the *puhA* gene and the DS *puhA* fragment. This allows for DNA either side of the ZF to be recognized and allelic exchange to take place. The arrows show the positions of primers 1-8 and L refers to a short 39bp linker section comprising of helix forming amino acids.](image)

**Figure S1.** Schematic illustration of the *puhA-zif268* DNA construct made to attach the *zif268* zinc finger DNA of *zif268* to the *puhA* gene from *Rps. palustris*. The stop codon of the *puhA* gene was removed in primers 1 and 2 and the *zif268* inserted between the *puhA* gene and the DS *puhA* fragment. This allows for DNA either side of the ZF to be recognized and allelic exchange to take place. The arrows show the positions of primers 1-8 and L refers to a short 39bp linker section comprising of helix forming amino acids.

**Table S1.** Sequences and features of primers (5'-3') used in the cloning of the *puhA-zif268* DNA construct.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>tegctgtatatggegttccgcgcgc</td>
<td>Zif268 forward primer, bold sequence corresponds to <em>puhA</em></td>
</tr>
<tr>
<td>2</td>
<td>aecgecagataccgagacctcgaacg</td>
<td><em>puhA</em> reverse primer, bold sequence corresponds to <em>zif268</em></td>
</tr>
<tr>
<td>3</td>
<td>gegeactctcgacagcccccgacctg</td>
<td>DS <em>puhA</em> forward primer, bold sequence corresponds to <em>zif268</em></td>
</tr>
<tr>
<td>4</td>
<td>ggctgtgcaaggtacctcatagct</td>
<td>Zif268 reverse primer, bold sequence corresponds to DS<em>puhA</em></td>
</tr>
<tr>
<td>5</td>
<td>catagattcggtgttgctccatgacacc</td>
<td><em>puhA</em> forward primer, with EcoRI site</td>
</tr>
<tr>
<td>6</td>
<td>aagettcccgatgtgtgatgtgac</td>
<td>DS <em>puhA</em> reverse primer with HindIII site</td>
</tr>
</tbody>
</table>

The *puhA* gene was amplified from *Rps. palustris* gDNA using primers 5 and 2 (listed in Table 1) and a 500bp directly downstream (DS) of the *puhA* gene was also amplified from gDNA using primers 3 and 6. The *zif268* DNA was obtained from Prof. Marshall Stark, University of Glasgow and was amplified using primers 1 and 4 1% Agarose gel electrophoresis of all of these PCR reactions are shown in Figure S2. DNA bands were excised from the gels and the DNA purified and eluted into 50μl using a QIAquick gel extraction kit (Qiagen).
Figure S2. Agarose gels showing amplification and assembly of the puhA-zif268 construct. a) Agarose gels for each of the individual pieces of the construct; the puhA gene from *Rps. Palustris* (795bp), the zif268 DNA binding domain (334bp) and puh DS (513bp) also from *Rps. palustris*. b) The individual fragments were then assembled by joining first the puhA and the zif268 (1129bp) and then finally joining the puh DS. This full puhA-zif268 construct (red arrow) at 1619bp was excised from the gel, purified and was ligated into suicide vector pK18mobsacB using HindIII and EcoRI restriction sites and confirmed by DNA sequencing (shown in Figure S3). For all gels the ladder used was 1kb DNA ladder from Promega. Two adjacent DNA fragments were attached together by adding 5µl of purified PCR product in a Phusion polymerase reaction without primers or polymerase at initial denaturation 95°C for 2min followed by 8 cycles of denaturation 95°C – 10 s, annealing 37°C – 20 s, extension 72°C – 1min, ending with a final extension of 5 mins. Outmost primers and polymerase were added and normal PCR amplification followed as directed by the polymerase protocol. The puhA-zif268 construct was ligated into the suicide vector pK18mobsacB [2] by EcoRI and HindIII restriction sites (plasmid obtained from Prof. Judy Armitage, University of Oxford) and transformed into *E.coli* JM109. The DNA constructs were verified by sequencing (shown in Figure S3).

**Figure S3.** Sequence confirmation of puhA-zif268 construct by MWG Eurofins. DNA construct for making the zfRC-LH1 strain of *Rps. palustris* was confirmed by sequencing at MWG Eurofins (http://www.eurofinsgenomics.eu/). The sequence illustration shows the puhA gene without stop codon (red), 39bp linker region (yellow), zif268 zinc finger DNA binding domain (blue) and the puhA sequence DS of the puhA gene (green). This DNA construct was ligated into the pK18mobsacB suicide vector using restriction enzyme HindIII and EcoRI which are shown in black.
Conjugative transfer

*PuhA-zif268* pK18mobsacB was transformed into chemically competent *E.coli* S-17-1 λ *pir* cells prior to conjugation and grown in liquid media aerobically with kanamycin for 19 hrs at 37ºC. *Rps. palustris* cells were grown in liquid media aerobically in the dark at 30ºC shaking for 16hrs. Conjugative transfer was set up by mixing 100μl: 900μl of the *E.coli: Rps.palustris* cell cultures and centrifuged for 6000rpm for 2 mins. The cell pellet was resuspended in 50μl of LB and dotted onto LB plate, dried and incubated for 6 hours at 37ºC as previously described. Bacterial plaques were scraped off the agar and resuspended in 200μl C-succinate media and plated onto agar of the same media supplemented with kanamycin to select for successful transconjugants. Kanamycin resistant colonies were grown in liquid culture and serial dilutions plated onto C-succinate plates supplemented with 10% sucrose. pK18mobsacB contains a *sacB* gene encoding levansucrase enzyme, which confers lethality to cells in the presence of sucrose. The presence selects for the successful transfer of the DNA construct into the genome by allelic exchange in a double recombination event. Successful colonies were further tested on duplicate grid plates for a loss of vector-mediated kanamycin resistance. Colony PCR was performed using primers designed to gDNA outside the region of allelic exchange. Presence of a longer amplified DNA seen by agarose gel electrophoresis indicated to addition of the zif268 on the *puhA* gene which was further confirmed by DNA sequencing.

Protein purification

Cells were harvested, broken and membranes purified as described previously. In brief, the cell cultures were centrifuged at 4000xg for 20mins and washed 3 times in 1x MES buffer. A pinch of MgCl₂ and DNase was added to harvested cells, and then broken by three passages through the French press at 9500 psi. Membranes were pelleted at 45,000rpm for 90 mins and resuspended in 20mM Tris (pH8) and the concentration adjusted to 0.1% LDAO. Membranes were solubilized using 1% LDAO stirring for an hour at RT and any unsolubilized material sedimented by centrifugation at 16,000g for 15 mins. The supernatant was layered onto sucrose gradients consisting of 0.8M, 0.6M, 0.4M and 0.2M sucrose in 20mM Tris (pH8) 0.1% LDAO and ultra-centrifuged at 45,000rpm for 16hrs at 4ºC. The lower band containing the RC-LH1 and was removed and purified on a DE52 anion exchange column. The purity of the protein sample was estimated by the ratio of protein absorption at 280nm to Bacteriochlorophyll at 878nm and eluted samples with a value higher than 1.9 were pooled. Protein samples were concentrated by centrifugation at 4000xg through an Amicon concentrator with a cut off at 50,000kDa. Protein samples were run on Invitrogen Novex SDS-PAGE gel according to the manufacturer’s instructions.

![Figure S4.](image)

Figure S4. a) SDS-PAGE of purified WT RC-LH1 complex and zfRC-LH1 WT bands for H, L and M show that the WT H subunit is ~ 29kDa. The zfRC-LH1 H-subunit is seen at 40kDa and indicated by a black box. This heavier band would correspond to the extra 11kDa of the zif268 binding domain. Also shown are the two α/β apoprotein bands of the LH1 at ~5/7 kDa respectively. b) The absorption spectra of the purified WT RC-LH1 complex (red) and zfRC-LH1 (black) normalized to the Q₉ peak at ~600nm.
MALDI-TOF Mass Spectrometry

A 3:1:2 mixture of formic acid/ water/ isopropyl alcohol was prepared and used to make a saturated solution of α-cyano-4-hydroxycinnamic acid (4HCCA). The mixture was vortexed and centrifuged at 7000rpm for 6 minutes to pellet any undissolved 4HCCA and supernant was removed to a fresh tube (Matrix 1). 1µl and 4µl of both zfRC-LH1 and WT RC-LH1 (OD10) was mixed with 15ul of Matrix 1 and vortexed. Samples were then centrifuged at 13000rpm to pellet aggregates. 2.5µl of each sample was air dried on a clean sample plate and MALDI-TOF carried out using Applied Biosystem Voyager System 4320. The zif268 tagged H-subunit showed a distinct peak at 39.2kDa compared to WT H-subunit observed at 27.3kDa.

Figure S5. MALDI-TOF for the WT RC-LH1 and zfRC-LH1. The L and the M subunits of the RC show up at the same correct sizes of 18kDa and 23kDa respectively. The WT H-subunit peak is present at 28kDa as previously documented. However the WT H-subunit is totally absent from the zfRC-LH1. There is an extra peak at 39kDa which would correspond to the addition of the zif268 DNA binding domain on the H-subunit. Smaller peaks represent fragments of the zif268 DNA binding domain formed during the mass spectrum measurements.

DNA binding gel shift assay

An 80-mer oligonucleotide (ODN80) incorporating a single zinc finger recognition site (highlighted in bold, Table S2) was designed and used in a DNA binding assay based on a previously reported protocol.[7] zfRC-LH1 protein was added to the 2x DNA binding buffer (500nM of 80bp dsDNA, 40mM Tris pH8, 40μM Zinc acetate, 4% Ficoll, 0.2% LDAO) at OD concentrations of 0.5, 1, 2.5 and 5 and incubated at RT for 30mins. Sample buffer and 10% DDM detergent supplied by the manufacturer were used as directed to aid protein running in native gel. Protein-DNA binding was assayed by running clear native gels (Invitrogen Native PAGE 4-16% Bis-Tris Gels) using the anode buffer in both chambers. The DNA stain used for the native gel was SYBR Green I nucleic acid stain (Invitrogen) diluted 10,000 x in TB buffer for 30mins. The gel was washed 3 times for 15 seconds in dH2O before visualised using transillumination scanning on a standard UV gel viewer. SimplyBlue Safe Stain (Life Technologies) was used for protein staining for 1 hour at RT with rotation and destained in water.

Table S2: 80 mer oligodeoxyribonucleotide used for gel shift assay (Fig. 2). Boldfaced sequences highlight zif268 binding site.

| DNA80 | 5'-AGCTTGCTCTCTGGATCCGACCTCTCATCTTTGCGTGCGGCTT |
| DNA80C | 5'-GCTGGGCATGCTATTGAATCATCATACTTTGAGCGAAGCT-3' |

S5
General Procedure of synthesis of DNA cyclooctyne conjugates 3-4

DNA 1-2 were purchased from Biotez, Berlin, Germany. Azidoundecyl trimethoxy silane<sup>8</sup> and 4-(cyclooct-2-yn-1-yl)oxy)butyl (2,5-dioxopyrrolidin-1-yl)<sup>9</sup> were prepared using reported procedures. Analytical RP-HPLC was performed at room temperature on an ULTIMAT 3000 Instrument (DIONEX). UV absorbance was measured using a photodiode array detector at 260. A Clarity Oligo RP C18 (4.6 × 250 mm, 5 µm) column was used for analytical RP-HPLC runs. The following HPLC gradient was used: 5% of B (0.1 M TEAAc, pH 7.6, 65% (v/v) MeCN (aq), held at 5 min. then increased to 90% of B over 20 min. Buffer A: 0.1 M TEAAc (aq), pH 7.6; Buffer B: 0.1 M TEAAc, pH 7.6, 65% (v/v) MeCN (aq).

**Table S3:** Sequences of hairpin oligodeoxyribonucleotides immobilized to glass slide. HEG = Hexaethyleneglycol.

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>DNA1</td>
<td><strong>Match</strong> (41 mer)</td>
<td>(5’-3’)</td>
</tr>
<tr>
<td>DNA 2</td>
<td><strong>Mismatch</strong> (41mer)</td>
<td>(5’-3’)</td>
</tr>
</tbody>
</table>

Amino-terminated DNA (1-2) (50 µL, 0.5 mM in water) was added to a NaHCO<sub>3</sub> solution (150 µL, 25 mM in water). This mixture was then added to a solution of 4-(cyclooct-2-yn-1-yl)oxy)butyl (2,5-dioxopyrrolidin-1-yl) carbonate (0.25 mg in 50 µL of acetonitrile). The reaction was stirred for 12 hours at room temperature. The reaction mixture was then separated using a GE Healthcare NAP-5 column (eluting with water). MALDI mass spectrum confirmed the formation of the product (3 - expected m/z: 14032.68, observed m/z: 14041.98, 4 - expected m/z: 13989.68, observed m/z: 14001.49). The DNA-cyclooctyne conjugates were used without any further purification.

**Figure S6.** MALDI-TOF mass spectra of DNA-cyclooctyne conjugates a) 3 - expected m/z: 14032.68, observed m/z: 14041.98 b) 4 - expected m/z: 13989.68, observed m/z: 14001.49.
Assessment of the Performance of Strain-Promoted Click conjugation with DNA (3-4)

To a CH$_3$CN:DMSO (1:1, 10 µL, 0.5 mM) solution of DNA (3-4) was added 50 eq. of 4-((3-azidopropyl)carbamoyl)-3,6-dichloro-2-(2,4,5,7-tetrachloro-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid in CH$_3$CN: DMSO (1:1, 25 µL). The two triazole regioisomers (5a) and (5b) from DNA 3 and (6a) and (6b) from DNA 4 were formed in 24 h (Scheme 1).

Figure S7. Denaturing PAGE of DNA sequences 1 – 6. a) UV illumination in the absence of SYBR-safe, highlighting fluorescein labelled click products DNA 5 and DNA 6. b) UV illumination after staining with SYBR-safe, revealing unlabeled sequences. Electrophoresis was carried out at 150 V in 20 % polyacrylamide gels with TBE buffer (pH 8.0) and 6 M urea. All samples were heated for 3 minutes at 90 °C in the presence of 3 M urea immediately prior to loading on the gel. Gels were imaged using UV illumination on standard gel documentation equipment.
Scheme 1. Synthesis of DNA cyclooctynes 3-4 and their conjugation with 4-((3-azidopropyl)carbamoyl)-3,6-dichloro-2-(2,4,5,7-tetrachloro-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid. Reagents & conditions: (i) 0.025M NaHCO₃ buffer, CH₃CN, RT, 12 hrs. (ii) 4-((3-azidopropyl)carbamoyl)-3,6-dichloro-2-(2,4,5,7-tetrachloro-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid, CH₃CN-DMSO, 24 hrs.
References


