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DNA-directed spatial assembly of photosynthetic light-harvesting proteins

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Sarah L. Henry[a], Jamie M. Withers,[b] Ishwar Singh[b,c], Jonathan M. Cooper[d], Alasdair W. Clark[d], Glenn A. Burley[b]*, Richard J. Cogdell[a]**

This manuscript describes the surface immobilization of a light-harvesting complex to prescribed locations directed by the sequence-selective recognition of duplex DNA. An engineered light-harvesting complex (RC-LH1) derived from *Rhodopseudomonas (Rps.) palustris* containing the zinc finger (ZF) domain *zif*268 was prepared. The *zif268* domain directed the binding of zfRC-LH1 to target double-stranded DNA sequences both in solution and when immobilized on lithographically defined micro-patterns. Excitation energy transfer from the carotenoids to the bacteriochlorophyll pigments within zfRC-LH1 confirmed that the functional and structural integrity of the complex is retained after surface immobilization.

Photosynthetic organisms are adept at both capturing solar energy and utilizing this energy to drive cellular metabolism.[1] The first step of the photosynthetic process involves absorption of solar energy by antenna light-harvesting modules (*e.g.,* LH2), followed by the channelling of this photonic energy towards a second membrane-bound light-harvesting protein (*e.g.,* RC-LH1) where charge separation occurs at a reaction centre (RC) housed within a lipid membrane. These steps require tight control over protein assembly within membrane-bound thylakoid stacks. This example of exquisite three-dimensional control potentially offers a blueprint for the construction of bio-inspired artificial light-harvesting assemblies for biofuel applications.[2] A key challenge that artificial photosynthesis faces is to both capture and harness sufficient amounts of light energy for practical applications. The organisation of monolayers of light-harvesting antenna and reaction centres onto electrodes to address step one of the process has previously been demonstrated.[3] These monolayers create a light-induced electrical current; however the amount of absorbed light is small and methods are now required to control *(i)* the spatial arrangement of light-harvesting complexes to prescribed locations along a surface (*i.e.,* two dimensional arrangement), and *(ii)* arrange light-harvesting complexes within a three-dimensional stack akin to the thylakoid stacks found in photosynthetic organisms. The DNA-directed self-assembly of non-natural components and functional materials holds considerable potential as an architecture to assemble functional materials in three-dimensional space.[4]

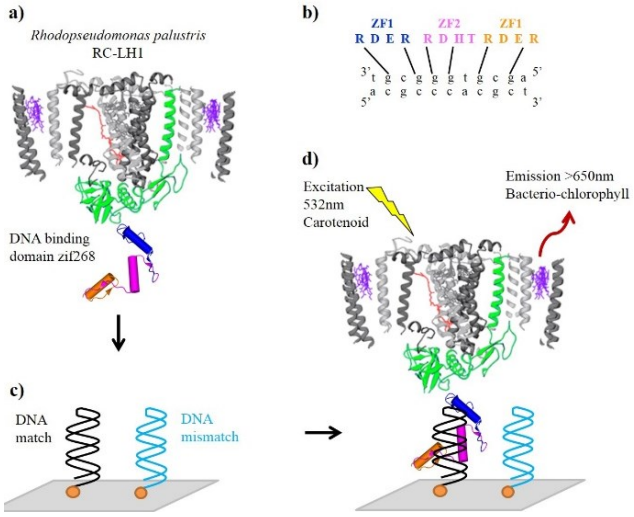

Fig. 1. (a) Structure of the RC-LH1 from *Rhodopseudomonas palustris* (grey) with the H-subunit highlighted in green (structure adapted from ref. 5). **(b)** An eleven bp DNA *zif268* recognition sequence. **(c)** Immobilization of cyclooctyne-tagged dsDNA to an azide-modified surface. **(d)** Excitation Energy Transfer (EET) was measured between the carotenoid pigment (in red) and bacteriochlorophyll (highlighted in purple) as a test for photosynthetic function.

[a] Dr. S. L. Henry, Prof. R. J. Cogdell.
Molecular Cell and Systems Biology, College of Medical, Veterinary and Life Sciences. University of Glasgow, Glasgow G12 8TA (UK)
E-mail: Richard.Cogdell@glasgow.ac.uk
[b] Dr Jamie M. Withers, Dr I. Singh, Dr. G. A. Burley.
Department of Pure and Applied Chemistry, University of Strathclyde. Glasgow G1 1XL (UK)
Email: glenn.burley@strath.ac.uk
[c] Present address: School of Pharmacy, College of Science, University of Lincoln.LN6 7TS (UK).
[d] Dr A. W Clark, Prof. J. M. Cooper,
School of Engineering, Biomedical Engineering, University of Glasgow, G12 8TL (UK).
Email: Alasdair.clark@glasgow.ac.uk
Electronic Supplementary Information (ESI) available: Experimental procedures and characterisation data.

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An excellent example of this is the positioning of fluorophores and larger photosynthetic components by binding to target single- or double-stranded DNA (dsDNA) sequences along and/or within larger DNA nanostructures.[6] This work describes the first step towards the development of DNA-directed light-harvesting assemblies immobilized on a surface by demonstrating the sequence selective binding of an engineered RC-LH1 complex to duplex DNA immobilized to a surface (Fig. 1).

We engineered an RC-LH1 complex (zfLH1-RC) derived from *Rhodopseudomonas (Rps.) palustris* that incorporated the well-characterised *zif268* DNA binding domain on the C-terminus of the H-subunit of the RC (Fig. 1a).[7] The *zif268* domain consists of three zinc finger motifs that bind to an eleven base-pair duplex DNA sequence (Fig. 1b) with nanomolar affinity.[8] Analysis by SDS-PAGE and MALDI-ToF confirmed the presence of the *zif268* tag. The spectral properties of the zfRC-LH1 showed no difference in the bacteriochlorophyll and carotenoid absorption compared to the wildtype, indicating that the structural and functional integrity of LH1-RC was not perturbed by the installation of *zif268*.

A DNA binding assay was used to test the ability of purified zfRC-LH1 to bind to a representative 80-mer oligodeoxyribonucleotide (ODN) containing the *zif268* target dsDNA sequence (Fig. 2). The DNA-stained native gel showed two distinct bands of DNA corresponding to free DNA and DNA in complex with zfRC-LH1 respectively (red arrows, Fig. 2a).[9]

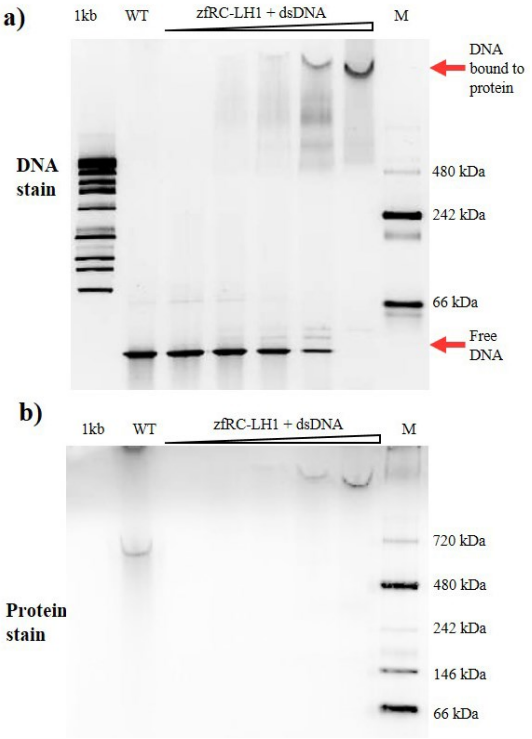

Fig. 2. Native gel electrophoretic analysis of zfRC-LH1 and stained for **(a)** DNA and **(b)** protein. zfRC-LH1 was incubated with 500nM 80bp DNA containing a single ZF binding sequence. 1kb: 1kb DNA ladder, WT: RC-LH1. zfRC-LH1 + dsDNA contains protein with an OD 0, 0.5, 1, 2.5 and 5. M: Nativemark protein marker (kDa). WT = wildtype.

The intensity of the band of reduced electrophoretic mobility increased as the concentration of zfRC-LH1 protein was increased. Concurrently, the intensity of the band corresponding to the free unbound DNA decreased as a function of increasing concentration of zfRC-LH1. The WT RC-LH1 did not show any additional DNA-containing bands, indicating that the wildtype does not bind to DNA. The difference in the molecular weight of the RC-LH1 complexes from the wildtype was most likely due to the addition of zif268 (Fig. 2b).

In order to test the robustness of zfRC-LH1 binding to the surface-immobilised target DNA sequence, DNA micro-patterns were fabricated on glass slides using a combination of electron-beam lithography and strain-promoted azide–alkyne cycloaddition (SPAAC).[10] Arrays of 2 µm squares were patterned by electron-beam lithography on a glass substrate using a PMMA (polymethylmethacrylate) resist. The exposed glass patterns were then functionalised with azidoundecyl trimethoxysilane.[11] A 41-mer hairpin DNA strand derivatised with a 5'-cyclooctyne group was then used to functionalise the azide-derivatised patterns *via* SPAAC.[10b] Finally, the DNA-functionalised glass surfaces were then spotted with zfC-LH1 and incubated overnight at 4 ˚C. After rinsing the excess protein with de-ionised water, the sample was sonicated for 10 minutes to minimise non-specific binding. Excitation of zfRC-LH1 derivatised surfaces at 532 nm resulted in fluorescent emission >650 nm only in those rows with DNA containing the *zif268* binding site (Fig. 3).

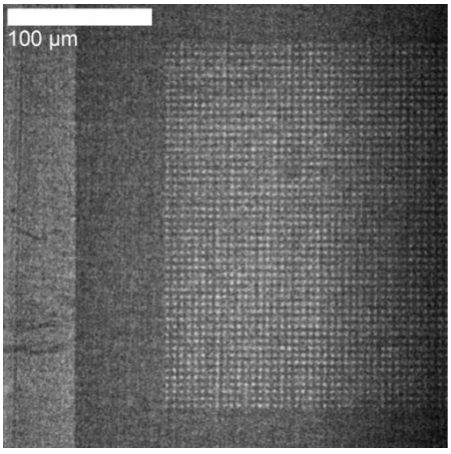

Fig. 3. Fluorescence microscopy image of zfRC-LH1 binding to DNA-functionalized glass surfaces. The DNA was patterned in a regular array of 2 µm squares, defined by electron-beam lithography. Excitation: 532 nm. Emission: > 650 nm. The fluorescence signal on the left of the picture relates to a large marker in order to aid array location.

This confirms that excitation energy transfer occurs from the carotenoids to the bacteriochlorophyll pigments and gives an unequivocal signature that the immobilization and sonication steps did not affect the LH capability of the RC-LH1 module.

Dip-pen-nanolithography (DPN) was then used to directly compare the sequence selectivity of zfRC-LH1 for immobilized DNA containing the matched target *zif268* sequence *versus* a one base-pair mismatch (Fig. 4a). A bright field image of the derivatised surface confirmed immobilization of both

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sequences *via* dipping a pen coated with cyclooctyne-tagged ODNs on a prescribed location on an azide-derivatised surface using SPAAC chemistry (Fig. 4b).[10b] The surface containing both matched and mismatched sequences was then incubated with a solution containing zfRC-LH1 (Fig. 4c). Consistent with the surfaces patterned by electron-beam lithography, fluorescence was observed only in the areas spotted with the fully matched DNA.

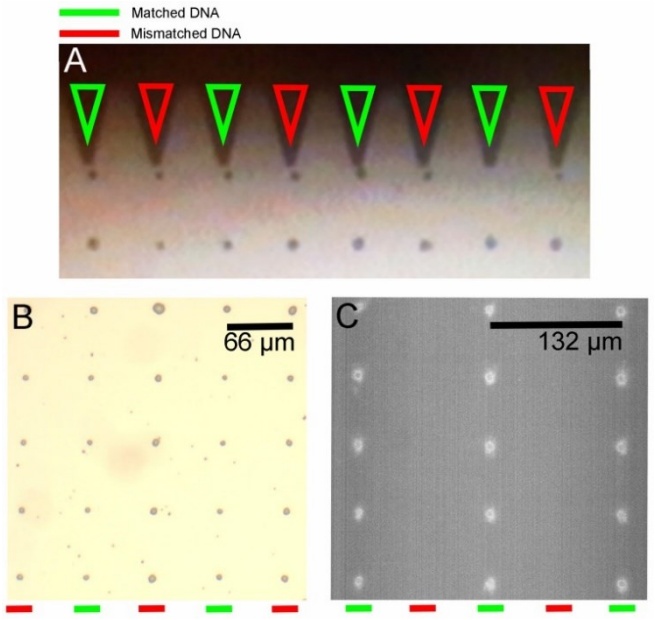

Fig. 4. Sequence-selective binding of zfRC-LH1 to matched and mismatched dsDNA using dual-sequence DPN. **(a)** DNA printing using pens to create alternate 5 µm spots of matched (green) and mismatched (red) DNA. **(b)** Bright field image of the printed DNA array. **(c)** Fluorescence image of the pattern after ZF-RC-LH1 binding to DNA. Each row is spaced 66µm apart. Excitation: 532 nm. Emission: > 650 nm.

We therefore conclude that the sequence selectivity of our zfRC-LH1 protein arises from the affinity and sequence selectivity of the *zif268* DNA binding domain grafted to the engineered RC-LH1.

In summary, we have reported the first example of an engineered light-harvesting protein exhibiting sequence-selective binding to target DNA sequences. The function of both the DNA binding and light-harvesting modules were unperturbed, thus creating a new synthetic biology-inspired approach for the spatial assembly of light-harvesting complexes to prescribed locations directed by a combination of lithographic micro-patterning and sequence-selective recognition of immobilized duplex DNA. Future work will now focus on directing the assembly of zfRC-LH1 into more sophisticated two- and three-dimensional DNA nanostructures in order to enhance the light-harvesting capabilities of the assemblies. Developments towards this goal are currently underway in our laboratories and will be reported in due course.

Experimental Section

Synthesis of zfRC-LH1: The *puhA* gene (Rps. palustris; NP_946894) encoded the H-subunit. Standard molecular cloning techniques were used to amplify *puhA* lacking the stop codon. A 39bp linker was attached followed by the *zif268* gene. DNA 500bp downstream (DS) of the *puhA* gene was amplified from gDNA and attached following the stop codon of the *zif268* tag *via* homologous recombination. The DNA construct was ligated into the suicide vector pK18mobsacB (see ESI for further details). The plasmid was transferred into *Rps. palustris* cells by conjugation and the *puhA-zif268* construct was exchanged with the WT allele in the gDNA of *Rps. palustris* by homologous recombination. Bacterial colonies containing the *puhA-zif268* were identified by PCR and bands confirmed by DNA sequencing (see ESI for further details). Bacterial cultures were grown anaerobically in the light (15µW m-1), and cells pelleted by centrifugation at 4000xg for 20mins. The harvested cells were broken by passage through a French Press cell and the membranes were isolated prior to solubilisation. ZF-RC-LH1 was purified by sucrose density gradient centrifugation.

DNA binding assays of ZF-RC-LH1: Electrophoretic Mobility Shift Assays were performed by mixing ZF-RC-LH1 with an 80bp dsDNA containing a single *zif268* target sequence in a binding buffer (500nM of DNA, 40mM Tris pH8, 40µM zinc acetate, 4% Ficoll, 0.2% LDAO). The mixture was incubated at RT for 30mins and analysed by native gel electrophoresis.

DNA functionalization of surfaces prepared by electron-beam lithography: Functionalised micro-surfaces were patterned using a Vistec VB6 electron-beam lithography tool and PMMA resist. After development, the exposed glass surfaces were modified with a 2% azidoundecyl trimethoxysilane solution in ethanol for 2 hours. The resist mask was then stripped using acetone and the remaining glass surface was modified with a 0.5% solution of PEG-silane (2-methoxy polyethyleneoxy propyl trimethoxysilane) in toluene for 30 minutes (a step designed to prevent non-specific binding of the DNA and the protein to the surface). Cyclooctyne-modified DNA (see ESI for further details) was spotted onto the patterns and incubated at 4 ˚C overnight. The sample was washed with PBS before prior to the addition of ZF-RC-LH1. Fluorescence measurements were performed using a Zeiss Axiovert 200M confocal microscope with a Zeiss LSM 5 Live laser scanning module, in conjunction with a 20x 0.5 NA objective lens.

DNA functionalization of surfaces prepared by dip-pen nanolithography: A NanoInk DPN5000 was used in conjunction with a multi-pen cantilever to spot the corresponding DNA sequence onto a glass substrate modified with azidoundecyl trimethoxysilane.[11] Using ink-wells matched to the inter-pen distance (spaced by 66 µm), the pens were loaded alternately with matched and mismatched DNA solutions (200 µM) suspended in PBS, with a 20% by volume addition of glycerol to aid the printing process. Spots of ~5 µm diameter were printed onto the surface in a square array with periodicity of 66 µm, each row created by bringing the pens into contact with the substrate for 0.5 seconds. The sample was incubated overnight at 4 ˚C, washed with PBS, spotted with ZF-RC-LH1 and incubated for a further overnight period at 4 ˚C. The sample was washed using the same sonication process as outlined previously.

Acknowledgements

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DNA directed binding of photosynthetic light-harvesting proteins**

Sarah L. Henry, Jamie M. Withers, Ishwar Singh, Jonathan M. Cooper, Alasdair W. Clark, Glenn A. Burley*, Richard J. Cogdell**

Supporting Information

Table of contents

Experimental procedures

Bacterial growth, strains and media

Rps. palustris was grown anaerobically in C-succinate media in light at 15 $\mu W.m^{-1}$ at 30ºC as described previously.[1] *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 37ºC 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.[2]

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.[3] Primer pairs were designed for Splice Overlap Extension (SOE) PCR, incorporating an overlap sequence of 18bp to attach two adjacent fragments.[4] 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.

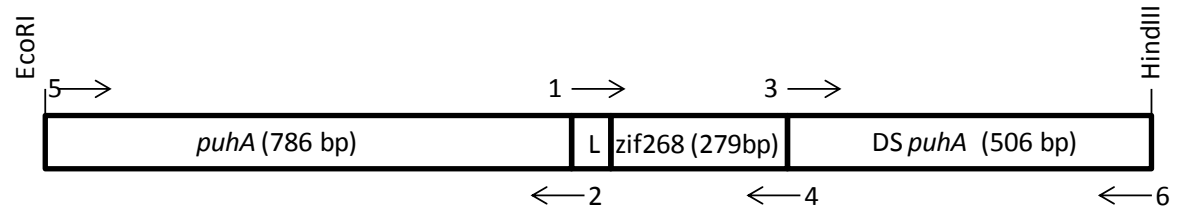

Figure S1. Schematic illustration of the *puhA-zif268* DNA construct made to attach the zif268 zinc finger DNA of zif268 to the *puhA* gene from *Rps. palustris*. The stop codon of the *puhA* gene was removed in primers 1 and 2 and the zif268 inserted between the *puhA* gene and the DS *puhA* fragment. 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.

Primer name	Primer sequence	Description
1	**tcgctcgta**tctggcggttccggcggc	Zif268 forward primer, bold sequence corresponds to puhA
2	**accgccaga**tacgagcgactccgaacg	puhA reverse primer, bold sequence corresponds to zif268
3	**gggtacctc**tcgacagccccagccagt	DS puhA forward primer, bold sequence corresponds to zif268
4	**ggctgtcga**gaggtaccctcatgagct	Zif268 reverse primer, bold sequence corresponds to DSpuhA
5	catagaattcgggtctgtcccatgcaacc	puhA forward primer, with EcoRI site
6	aagctttccggatgattgatgtacca	DS puhA reverse primer with HindIII site

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 1and 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).

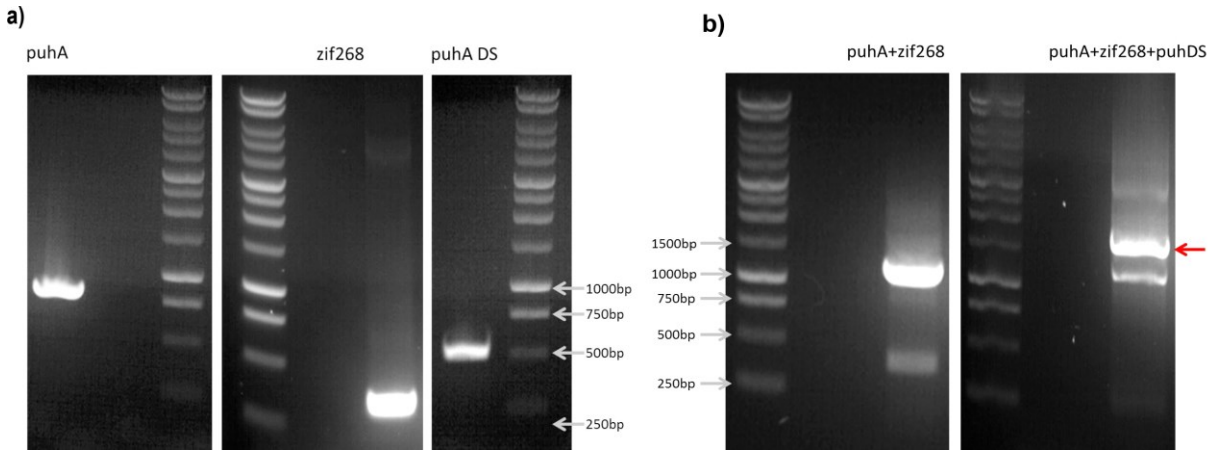

Figure S2. Agarose gels showing amplification and assembly of the puhA-zif268 construct. **a)** Agarose gels for each of the individual pieces of the construct; the puhA gene from *Rps. Palustris* (795bp), the zif268 DNA binding domain (334bp) and puh DS (513bp) also from *Rps. palustris*. **b)** The individual fragments were then assembled by joining first the puhA and the zif268 (1122bp) and then finally joining the puhDS. 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 95ºC for 2min followed by 8 cycles of denaturation 95ºC – 10 s, annealing 37ºC – 20 s, extension 72ºC – 1min, ending with a final extension of 5 mins. Outermost 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).

catagaattcGGGTCTGTCCCATGCAACCCGGAGCGTATTTGGACTTAGCACAGGTTACCCTGTACGTATTTTGGA
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GCGCGTGCTGCTTCAGATGGGCGTGGCGCTGCCGATCACGATCAACATCCCGTTGACCCGGATCGCCAACGCCGACCTCCGCCAAAATCG
TGACGGCAGCGGCGATATCCCCCTGCGGATCATCGACGACAAGCGCGCATCCTACGTTCTGCTGTGGCCGCATGTGCGGCCTTGGTACAT
CAATCATCCGGAaagcttaagt

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 sites 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.[5] 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.[1] In brief, the cell cultures were centrifuged at 4000xg for 20mins and washed 3 times in 1x MES buffer. A pinch of $MgC1_2$ 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 OD at 864nm =50. Membranes were solubilized using 1% LDAO stirring for an hour at RT and any unsolubalized 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.

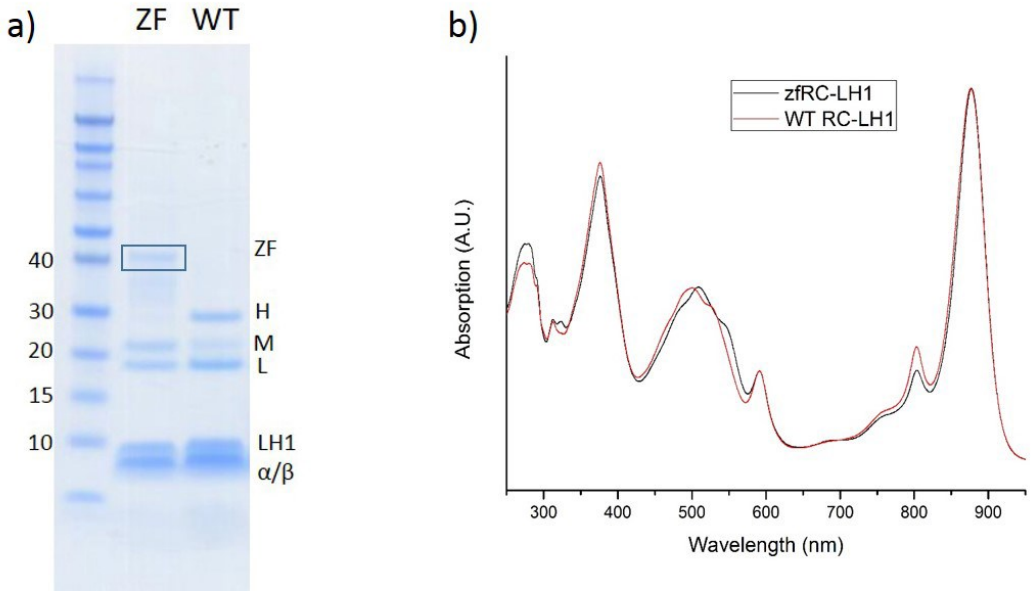


Figure S4. **a)** SDS-PAGE of purified WT RC-LH1 complex and zfRC-LH1 WT bands for H, L and M show that the WT H subunit is ~ 29kDa. The zfRC-LH1 H-subunit is seen at 40kDa and indicated by a black box. This heavier band would correspond to the extra 11kDa of the zif268 binding domain. Also shown are 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 Q_x 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.

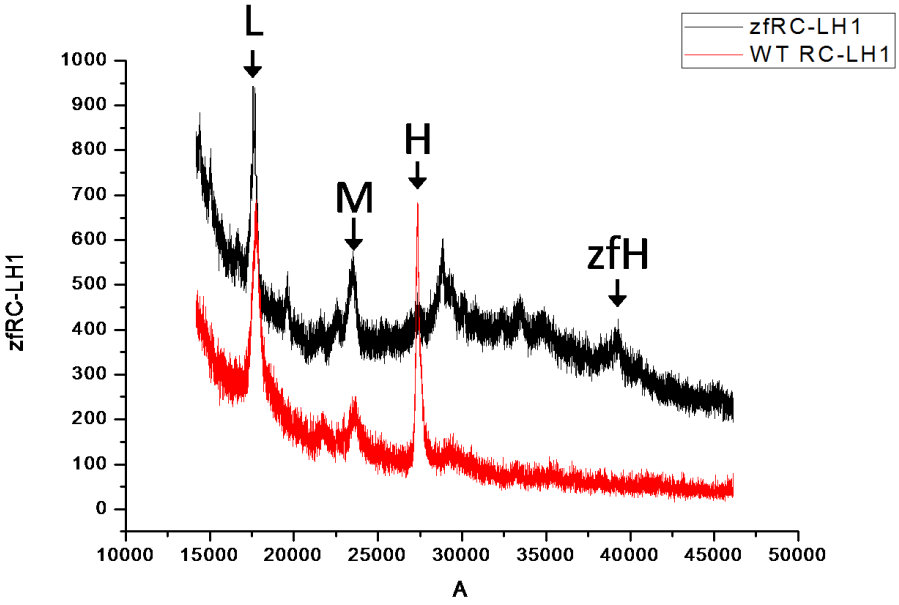


Figure S5. MALDI-TOF for the WT RC-LH1 and zfRC-LH1. The L and the M subunits of the RC show up at the same correct sizes of 18kDa and 23kDa respectively. The WT H-subunit peak is present at 28kDa as previously documented. However the WT H-subunit is totally absent from the zfRC-LH1. There is an extra 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)[6] was designed and used in a DNA binding assay were 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 *zif*268 binding site.

	Sequence (5' → 3')
DNA80	5'-AGCTTGCCTCTCTGCGATCACTCATACTTT**GCGTGGGCGT**T CTCTAGAGCTTAAGTATGATTCAATAGCATTGAGCCAAGC-3'
DNA80C	5'-GCTTGGCTCAATGCTATTGAATCATACTTAAGCTCTAGAG **AACGCCCACGC**AAAGTATGAGTGATCGCAGAGAGGCAAGCT-3'

General Procedure of synthesis of DNA cyclooctyne conjugates 3-4

DNA **1-2** were purchased from Biotez, Berlin, Germany. Azidoundecyl trimethoxy silane[8] and 4-(cyclooct-2-yn-1-yloxy)butyl (2,5-dioxopyrrolidin-1-yl)[9] 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.

DNA1	**Match** (41 mer)	(5`-3`)	Aminohexyl(HEG)$_3$ –CTT TTT TGC GTG GGC GTT TTT TTA AAC GCG CAC GCA AAA AA
DNA 2	**Mismatch** (41mer)	(5`-3`)	Aminohexyl(HEG)$_3$ –CTT TTT TTA CGT GGC ATT TTT TTA AAT GCC ACG TAA AAA AA

Amino-terminated DNA (**1-2**) (50 µL, 0.5 mM in water) was added to a $NaHCO_3$ solution (150 µL, 25 mM in water). This mixture was then added to a solution of 4-(cyclooct-2-yn-1-yloxy)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.

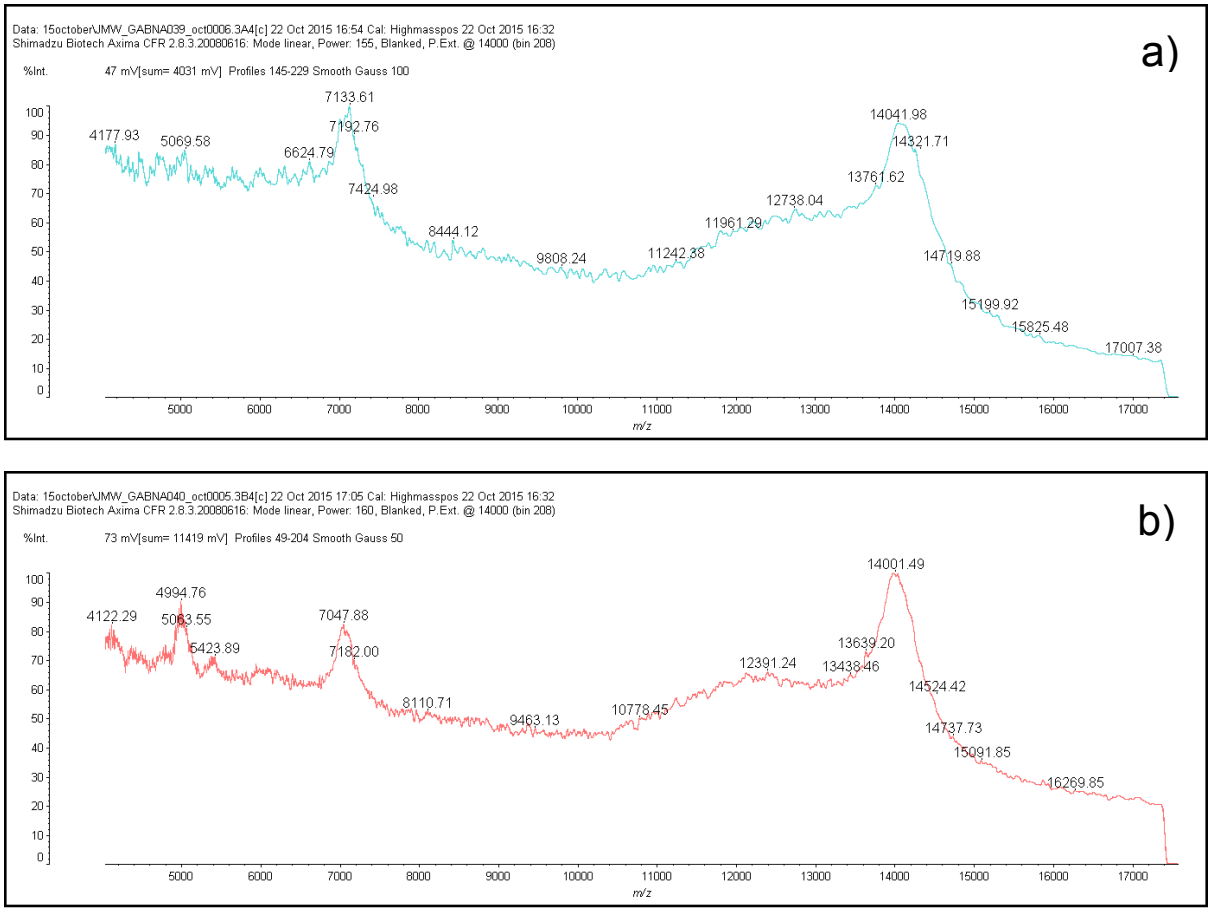


Figure S6. MALDI-TOF mass spectra of DNA-cyclooctyne conjugates a) **3** - expected m/z: 14032.68, observed m/z: 14041.98 b) **4** - expected m/z: 13989.68, observed m/z: 14001.49.

Assessment of the Performance of Strain-Promoted Click conjugation with DNA (3-4)

To a CH_3CN: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_3CN: 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).

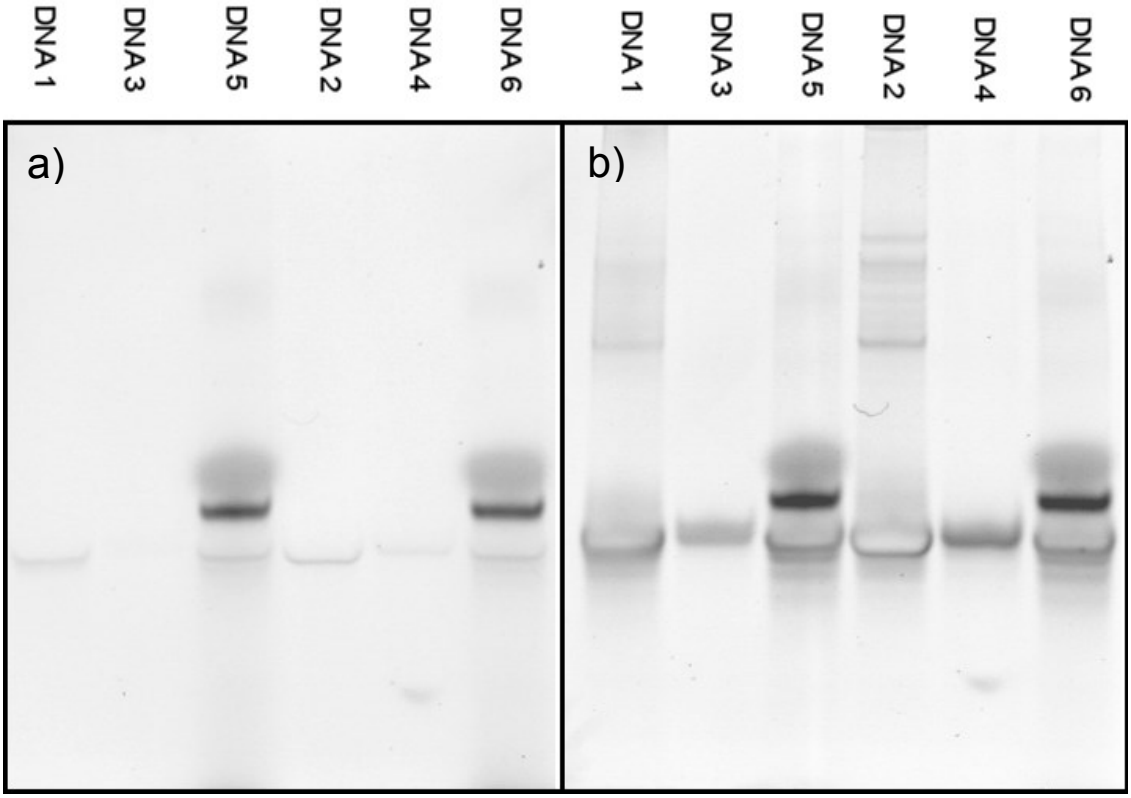
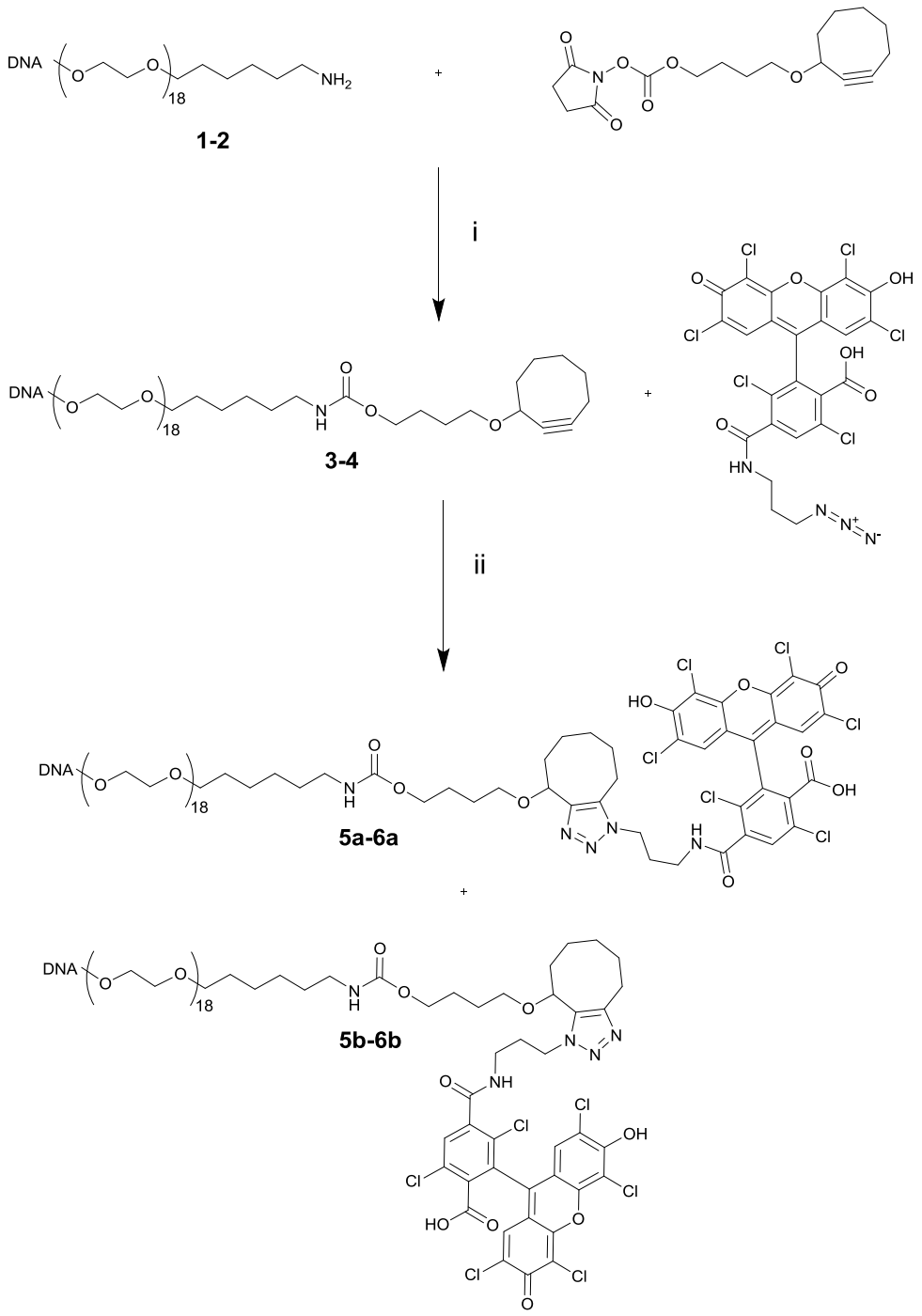


Figure S7. Denaturing PAGE of DNA sequences **1 – 6**. **a)** UV illumination in the absence of SYBR-safe, highlighting fluorescein labelled click products DNA **5** and DNA **6**. **b)** UV illumination after staining with SYBR-safe, revealing unlabeled sequences. Electrophoresis was carried out at 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 $NaHCO_3$ buffer, CH_3CN, 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, CH_3CN-DMSO, 24 hrs.

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