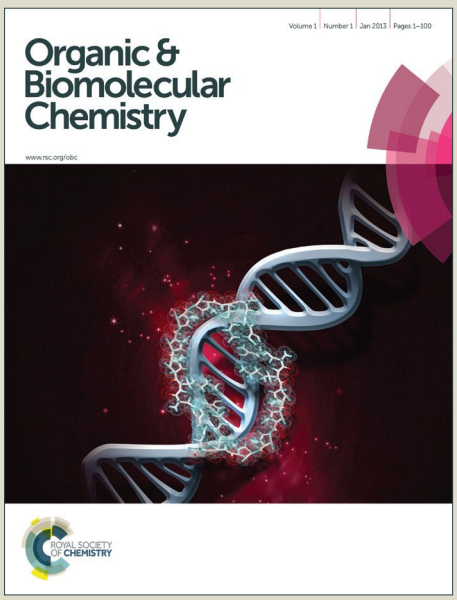
# Organic & Biomolecular Chemistry

Accepted Manuscript





ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Journal Name

COMMUNICATION

# DNA-directed spatial assembly of photosynthetic light-harvesting proteins

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

*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]

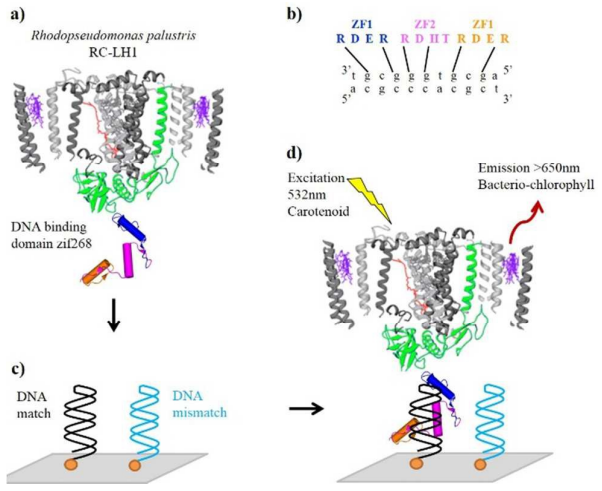



**Fig. 1. (a)** Structure of the RC-LH1 from *Rhodopseudomonas palustris* (grey) with the H-subunit highlighted in green (structure adapted from ref. 5). **(b)** An eleven bp DNA *zif268* recognition sequence. **(c)** Immobilization of cyclooctyne-tagged dsDNA to an azide-modified surface. **(d)** Excitation 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.

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]

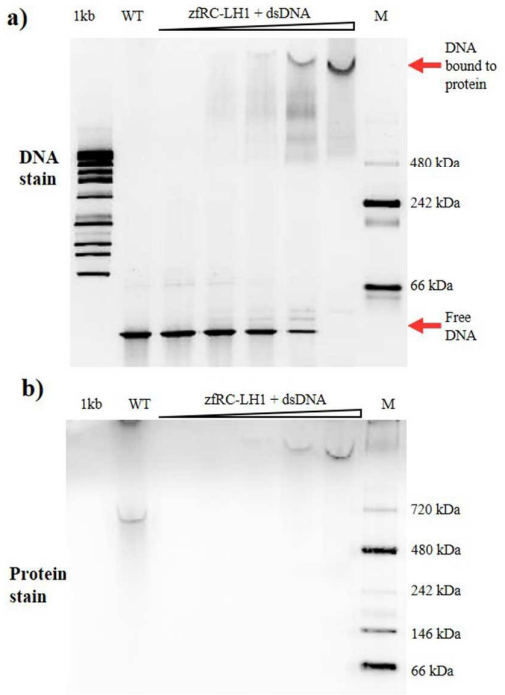



**Fig. 2.** Native gel electrophoretic analysis of zfRC-LH1 and stained for **(a)** DNA and **(b)** protein. zfRC-LH1 was incubated with 500nM 80bp DNA containing a single ZF binding sequence. 1kb: 1kb DNA ladder, WT: RC-LH1. zfRC-LH1 + dsDNA contains protein with an OD 0, 0.5, 1, 2.5 and 5. M: Nativemark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

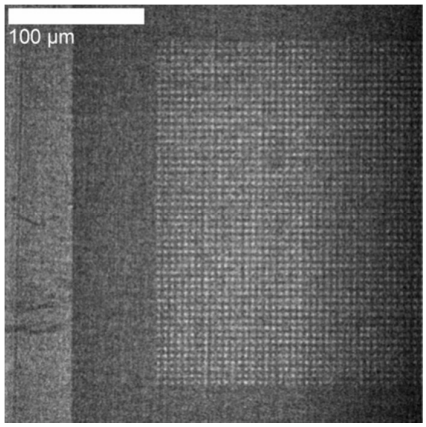



**Fig. 3.** Fluorescence microscopy image of zfRC-LH1 binding to DNA-functionalized glass surfaces. The DNA was patterned in a regular array of 2 µm squares, defined by electron-beam lithography. Excitation: 532 nm. Emission: > 650 nm. The fluorescence signal on the left of the picture relates to a large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

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.

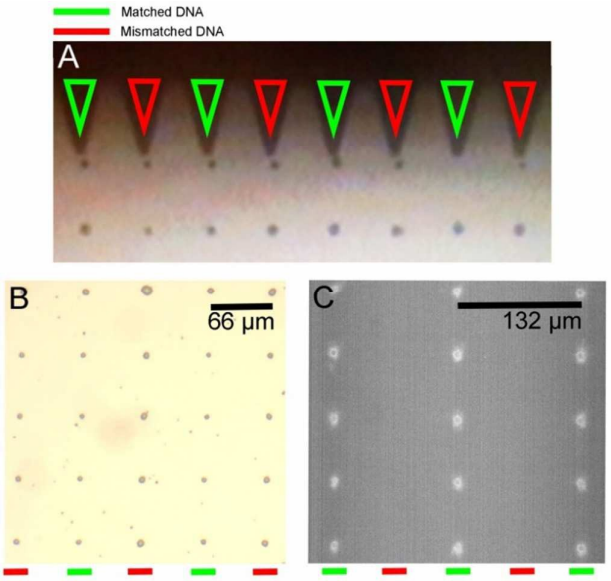



**Fig. 4.** Sequence-selective binding of zfRC-LH1 to matched and mismatched dsDNA using dual-sequence DPN. **(a)** DNA printing using pens to create alternate 5 µm spots of matched (green) and mismatched (red) DNA. **(b)** Bright field image of the printed DNA array. **(c)** Fluorescence image of the 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

SLH and RJC were supported by funding from Human Frontier of Science Program. AWC was supported by the Royal Academy of Engineering (grant number 10216/103). This research was supported as a part of the Photosynthetic Antenna Research Centre (PARC), an Energy Frontier Research Centre funded by the DOE, Office of Science, Office of Basic Energy Sciences under Award Number DE-SC 0001035.

## References


1. R. J. Cogdell, A. Gall, J. Köhler, *Quart. Rev. Biophys.*, 2006, **39**, 227-324.

2. (a)K. Kalyanasundaram and M. Graetzel, *Curr. Opin. Biotech.*, 2010, **21**, 298-310; (b) D. Gust, T. A. Moore and A. L. Moore, *Acc. Chem. Res.*, 2009, **42**, 1890-1898; (c) R. E. Blankenship, D. M. Tiede, J. Barber, G. W. Brudvig, G. Fleming, M. Ghirardi, M. R. Gunner, W. Junge, D. M. Kramer, A. Melis, T. A. Moore, C. C. Moser, D. G. Nocera, A. J. Nozik, D. R. Ort, W. W. Parson, R. C. Prince and R. T. Sayre, *Science*, 2011, **332**, 805-809.

3. (a) G. J. Magis, J. D. Olsen, N. P. Reynolds, G. J. Leggett, C. N. Hunter, T. J. Aartsma and R. N. Frese, *Photochem. Photobiol.*, 2011, **87**, 1050-1057; (b) S. Yajima, R. A. Furukawa, M. Nagata, S. Sakai, M. Kondo, K. Iida, T. Dewa and M. Nango, *App. Phys. Lett.*, 2012, **100**; (c) S. Sakai, T. Noji, M. Kondo, T. Mizuno, T. Dewa, T. Ochiai, H. Yamakawa, S. Itoh, H. Hashimoto and M. Nango, *Langmuir*, 2013, **29**, 5104-5109; (d) A. Sumino, T. Dewa, M. Kondo, T. Morii, H. Hashimoto, A. T. Gardiner, R. J. Cogdell and M. Nango, *Langmuir*, 2011, **27**, 1092-1099; (e) M. Kondo, Y. Nakamura, K. Fujii, M. Nagata, Y. Suemori, T. Dewa, K. Iida, A. T. Gardiner, R. J. Cogdell and M. Nango, *Biomacromolecules*, 2007, **8**, 2457-2463; (f) M. Escalante, A. Lenferink, Y. Zhao, N. Tas, J. Huskens, C. N. Hunter, V. Subramaniam and C. Otto, *Nano Lett.*, 2010, **10**, 1450-1457; (g) M. Escalante, Y. Zhao, M. J. W. Ludden, R. Vermeij, J. D. Olsen, E. Berenschot, C. N. Hunter, J. Huskens, V. Subramaniam and C. Otto, *J. Am. Chem. Soc.*, 2008, **130**, 8892-8893; (h) N. P. Reynolds, S. Janusz, M. Escalante-Marun, J. Timney, R. E. Ducker, J. D. Olsen, C. Otto, V. Subramaniam, G. J. Leggett and C. N. Hunter, *J. Am. Chem. Soc.*, 2007, **129**, 14625-14631; (i) R. Das, P. J. Kiley, M. Segal, J. Norville, A. A. Yu, L. Wang, S. A. Trammell, L. E. Reddick, R. Kumar, F. Stellacci, N. Lebedev, J. Schnur, B. D. Bruce, S. Zhang and M. Baldo, *Nano Lett.*, 2004, **4**, 1079-1083.

4. (a) N. C. Seeman, *Ann. Rev. Biochem.*, 2010, **79**, 65-87; (b) F. Zhang, J. Nangreave, Y. Liu and H. Yan, *J. Am. Chem. Soc.*, 2014, **136**, 11198-11211; (c) W. M. Shih and C. Lin, *Curr. Opin. Struct. Biol.*, 2010, **20**, 276-282; (d) W. Su, V. Bonnard and G. A. Burley, *Chem. Eur. J.*, 2011, **17**, 7982–7991.

5. A. W. Roszak, T. D. Howard, J. Southall, A. T. Gardiner, C. J. Law, N. W. Isaacs and R. J. Cogdell, *Science*, 2003, **302**, 1969-1972.

6. (a) I. H. Stein, C. Steinhauer and P. Tinnefeld, *J. Am. Chem. Soc.*, 2011, **133**, 4193–4195; (b) M. Heilemann, R. Kasper, P. Tinnefeld and M. Sauer, *J. Am. Chem. Soc.*, 2006, **128**, 16864-16875; (c) M. Heilemann, P. Tinnefeld, G. S. Mosteiro, M. G. Parajo, N. F. Van Hulst and M. Sauer, *J. Am. Chem. Soc.*, 2004, **126**, 6514-6515; (d) P. K. Dutta, S. Levenberg, A. Loskutov, D. Jun, R. Saer, J. T. Beatty, S. Lin, Y. Liu, N. W. Woodbury and H. Yan, *J. Am. Chem. Soc.*, 2014, **136**, 16618-16625; (e) P. K. Dutta, R. Varghese, J. Nangreave, S. Lin, H. Yan and Y. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 11985-11993.

7. N. P. Pavletich and C. O. Pabo, *Science*, 1991, **252**, 809-817.

8. (a) E. Nakata, F. F. Liew, C. Uwatoko, S. Kiyonaka, Y. Mori, Y. Katsuda, M. Endo, H. Sugiyama and T. Morii, *Angew. Chem. Intl. Ed.*, 2012, **51**, 2421-2424; (b) M. Elrod-Erickson and C. O. Pabo, *J. Biol. Chem.*, 1999, **274**, 19281-19285.

9. I. Wittig, M. Karas and H. Schägger, *Molec. Cell. Prot.*, 2007, **6**, 1215-1225.

10. (a) J. C. Jewett and C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, **39**, 1272-1279; (b) I. Singh, C. Wendeln, A. W. Clark, J. M. Cooper, B. J. Ravoo and G. A. Burley, *J. Am. Chem. Soc.*, 2013, **135**, 3449-3457.

11. N. Balachander and C. N. Sukenik, *Langmuir*, 1990, **6**, 1621-1627.