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DNA-directed spatial assembly of photosynthetic light-harvesting proteins

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 *Rhodospseudomonas (Rps.) palustris* containing the zinc finger (ZF) domain *zif268* 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 *Rhodospseudomonas 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

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 *Rhodospseudomonas (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 $^{\circ}\text{C}$. 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

Figure 1 consists of three panels (A, B, and C) showing DNA microarrays. Panel A is a fluorescence image of a microarray with a legend indicating 'Matched DNA' (green) and 'Mismatched DNA' (red). The array shows a series of spots, with some spots appearing green and others red. Panel B is a fluorescence image of a microarray with a scale bar of 66 μm. Panel C is a fluorescence image of a microarray with a scale bar of 132 μm. The arrays show a grid of spots, with some spots appearing green and others red.

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.

Experimental Section

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 dip-pen nanolithography: A Nanolnk 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 $^{\circ}\text{C}$, washed with PBS, spotted with ZF-RC-LH1 and incubated for a further overnight period at 4 $^{\circ}\text{C}$. The sample was washed using the same sonication process as outlined previously.

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