Experimental report

17/09/2018

Proposal: 8-02-752 **Council:** 4/2015

Title: Neutron Reflectometry Study of DNA-directed spatial assembly of photosynthetic light-harvesting proteins

Research area: Biology

This proposal is a new proposal

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Samples: Lipids
DNA
Recombinant Light Harvesting protein complex

Instrument	Requested days	Allocated days	From	To
D17	2	1	04/07/2018	05/07/2018

Abstract:

One of the key challenges for artificial photosynthesis is to optimize the capacity for light-harvesting (LH). We have recently developed a new method for the assembly of LH complexes onto DNA. Structural DNA nanotechnology holds considerable potential in this regard because the programmability and fidelity of DNA base-pairing provides access to a large variety of available DNA structures
The LH complexes have however not yet been organized into higher order assemblies, due to our limited ability to investigate LH spatial positioning in two- and eventually three-dimensions. We therefore propose to use Neutron Reflectometry (NR) to reveal the layered structure of designed DNA/LH complexes. The resultant data would enable further, more rational and informed designs of more complex LH/DNA assemblies.

Neutron Reflectometry Study of DNA-directed spatial assembly of photosynthetic light-harvesting proteins

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Background & goal

A key challenge for the field of artificial photosynthesis is the directed assembly of Light-harvesting complexes (LHC) with nanoscale spatial control in order to understand the molecular determinants of enhancing the efficiency of LH. A number of artificial arrays have been developed in which LH antenna and reaction centers (RC) have been organized onto electrodes in 2 dimensions to create a light-induced electrical current.[1] However, this approach suffers from an inherent limitation; specifically, the amount of electricity produced will always be small as a single layer of photosynthetic complexes has a very limited ability to absorb light. The programmability of DNA nanostructures provides the capacity for developing nanodevices that can help answer biophysical questions and solve a multitude of once challenging problems.[2] We have recently developed a new method for the assembly of LH complexes using a DNA binding domain (zif268) as a tag to allow for sequence selective binding to DNA. [3] However, the spatial arrangement of LH complexes into higher order assemblies still remains a challenge.

In parallel to this research, we recently developed a new method for immobilising DNA into defined patterns, with sub-micron resolution, by taking advantage of the fluorous effect.[4] This fluorous-directed immobilization is fully reversible, enabling directed surface patterning, regeneration, and re-patterning of surfaces without any associated degradation of immobilization efficiency or disruption of Watson-Crick base-pairing. We have designed and optimised the synthesis of a fluorous-DNA (R_F-DNA) suite varying the fluorous content (R_F-DNA, $(R_F)_2$-DNA, $(R_F)_4$-DNA). The development and future combination of our novel techniques will allow for the distinct arrangement of LHC in both two and three dimensions and will provide the field of synthetic photosynthesis with a unique and robust engineering tool.

Neutron Reflectometry (NR) offers essential information to reveal the layered structure of designed DNA/LH complexes. The study address the analysis of a complex multilayer system compose by, fluorous monolayer, fluorous-DNA (single strand), complementary DNA strand and finally the protein. Such a complex system needs a sequential study strategy, increasing the level of complexity stepwise. Prior to protein immobilisation it was crucial to determine the structural features of R_F-DNA on the fluorous monolayer and its influence on hybridisation process. The resultant data would enable further, more rational and informed designs of more complex DNA/LH complexes.

Experimental details

Specular neutron reflectivity (SNR) experiments were performed to study the interfacial structure of two different samples of fluorous DNA (R_F-DNA and $(R_F)_4$-DNA) on a fluorinated surface. The SNR measurements were made on the time of flight (TOF) D17 reflectometer at the ILL, Grenoble, France. R_F-DNA and $(R_F)_4$-DNA systems were deposited on highly polished 5x5 cm^2 fluorinated silicon blocks by direct injection of the sample into the solid-liquid cell. 5x5 cm^2 standard silicon block was used as a negative control. Data were collected at two incident angles 0.8 and 3.0. Calibration of D17 was performed using pure D_2O in contact with the silicon crystal coated with Heptadecafluoro-1, 1, 2, 2-tetrahydrodecyl trimethoxysilane (Fluorochem). Backgrounds were subtracted from the data by the simultaneous acquisition of

off-specular data for each measurement on the area detector. No off-specular scattering was observed for all the samples under the conditions of the experiment. All SNR experiments were performed at a temperature of 20°C by using a thermostatic bath.

Samples were prepared at the ILL using PSCM lab facilities. Freeze-dried 100 mM buffer phosphate pH 7.4 with 300 mM NaCl was dissolved in D_2O and H_2O respectively, and shaken for 30 minutes at room temperature. Subsequently, R_F-DNA and $(R_F)_4$-DNA dried samples were dissolved in the appropriated buffer volume to obtain a 5 μM solution. Next, samples were vortexed and concentration was measured. Same protocol was used to prepare DNA complementary strand (Eurogentec) solution.

Then, SNR profiles of R_F-DNA and $(R_F)_4$-DNA were obtained using two different H/D contrasts, namely D_2O as shown Figure 1 and H_2O (results not shown for clarity). We performed a full-Q structural NR analysis of the fluorous DNA layer, at two different compositions, with the aim to evaluate the differences in thickness and interfacial density at two different surface pressure values for each composition. SNR profiles were acquired sequentially. First measurement was recorded to obtain the profile of fluorous surface. Then, R_F-DNA and $(R_F)_4$-DNA were immobilized on the fluorous surface, and finally, complementary DNA strand was injected to study changes on the SNR profile after hybridization. Both sets of experiments were performed with two different samples of fluorous-DNA bearing one or four fluorous tails, R_F-DNA and $(R_F)_4$-DNA (Only plotted $(R_F)_4$-DNA for clarity).
The SNR profiles plotted in Figure 1 are being now analyzed by the Abeles matrix method using Motofit[5] assuming a stratified structure including the air, the fluorous-DNA layer and the Si/SiO_2 substrate. To find the parameters that show the most realistic model, the SNR data obtained for the two contrasts at each experimental condition were simultaneously fitted with a genetic algorithm and refined with a Levenberg-Marquardt optimization. SLD were calculated based on previous molecular composition of the structures.

Results

The main goal of the first experiment was to use SNR to compare the immobilisation and subsequent complementary DNA hybridisation properties of two different samples of fluorous-DNA, bearing one and four fluorous tails (R_F-DNA and $(R_F)_4$-DNA). Key questions to address are, whether fluorous density influences in the immobilisation process and fluorous-DNA surface density, as well as describe the structural features for both, R_F-DNA and $(R_F)_4$-DNA, when immobilised on the fluorous monolayer.
As it can be observed on Figure 1, there is a clear change in the SNR profiles in D_2O from the functionalized fluorous substrate with respect to the bare Si/SiO_2 substrate. Furthermore, subsequent immobilisation of both samples (only $(R_F)_4$-DNA shown for clarity) and DNA hybridisation was also observed by a change in the NR profile. However, hybridisation step did not show a pronounce effect on the NR signal.
Further data analysis is currently on-going and will allow us to determine the relative positions of the fluorous tails within the fluorous monolayer, as well as the DNA position for both samples R_F-DNA and $(R_F)_4$-DNA. We also expect a difference in fluorous-DNA surface density in both samples due to the increase in fluorous density in the molecule. Consequently, the surface density could have an influence into the complementary DNA hybridisation. DNA confinement due to surface immobilisation and molecular crowding prevents hybridisation.[6]

Conclusions.

We collected several complete NR datasets of R_F-DNA and $(R_F)_4$-DNA (experiments 8-02-764 and 8-02-752). Analysis is currently on-going, therefore only preliminary qualitative conclusions can be made. The initial analysis clearly demonstrated that structural changes could be directly observed by neutron reflectometry (Figure 1). After further analysis we expect to provide a quantitative characterization of the interfacial thickness, surface density and relative orientation of the different system components on the solid/liquid interface, rendering more rational and informed designs for the future introduction of the Light Harvesting complexes.

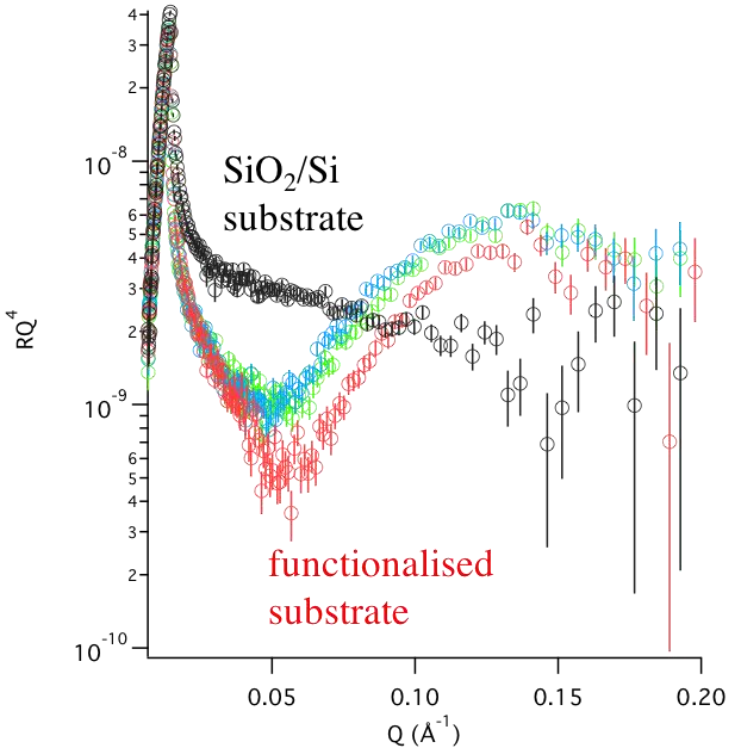

Figure 1. Full-Q Specular Neutron Reflectometry profile of the functionalised substrate (red), $(R_F)_4$-DNA in D2O (green) and the complementary DNA strand (blue).

References

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