View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

FEBS Letters 586 (2012) 2157–2163

journal homepage: www.FEBSLetters.org

Review

A Synthetic Biology Project – Developing a single-molecule device for screening drug–target interactions

Keith Firman, Luke Evans, James Youell *

IBBS Biophysics Laboratories, School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth PO1 2DY, United Kingdom

ARTICLE INFO

Article history:
Received 13 December 2011
Revised 31 January 2012
Accepted 31 January 2012
Available online 8 February 2012

Edited by Thomas Reiss and Wilhelm Just

Keywords:
Molecular motor
Magnetoresistive device
Single molecule sensor
Drug–target interaction
DNA modifying enzyme

ABSTRACT

This review describes a European-funded project in the area of Synthetic Biology. The project seeks to demonstrate the application of engineering techniques and methodologies to the design and construction of a biosensor for detecting drug–target interactions at the single-molecule level. Production of the proteins required for the system followed the principle of previously described "bioparts" concepts (a system where a database of biological parts – promoters, genes, terminators, linking tags and cleavage sequences – is used to construct novel gene assemblies) and cassette-type assembly of gene expression systems (the concept of linking different "bioparts" to produce functional "cassettes"), but problems were quickly identified with these approaches. DNA substrates for the device were also constructed using a cassette-system. Finally, micro-engineering was used to build a magnetoresistive Magnetic Tweezer device for detection of single molecule DNA modifying enzymes (motors), while the possibility of constructing a Hall Effect version of this device was explored. The device is currently being used to study helicases from Plasmodium as potential targets for anti-malarial drugs, but we also suggest other potential uses for the device.

© 2012 Federation of European Biochemical Societies. Published by Elsevier B.V.
Open access under CC BY-NC-ND license.

1. Introduction

One definition of Synthetic Biology is "*the application of engineering principles to the study of the fundamental components of biology*", but there are major problems associated with this basic premise – biological systems are very different from electronic systems, or chemical systems, and new combinations do not always behave as expected. One example of this *engineering styled approach*, applied to protein production, is the use of "bioparts"[1] from a database and the construction of novel combinations of these "bioparts" to produce the required proteins. The "bioparts" consist of specific gene sequences, promoters, terminators, tag systems for affinity purification and cleavage sites that allow release of the required protein. Each of these "bioparts" can be joined using a simple, identical system in a cassette-like manner allowing a potentially large number of different combinations. Such a system is often commercially available as a kit-based expression system (e.g. ATG: biosynthetics[2]). However, when this concept is applied to the assembly of novel components of a gene expression system, the production of novel proteins from any specific combination of gene and promoter is not guaranteed [1]. Problems usually arise from the unexpected loss of protein solubility. However, another view of this definition of Synthetic Biology is the use of biological components in the construction of useful devices, such as biosensors, using modular approaches associated with engineering principles. When such techniques are combined with the concepts of nanotechnology (single molecule manipulation and construction on a sub-100 nm scale), truly exciting and useful devices can be imagined.

We first outlined the construction of such a device following our studies with the Type I Restriction-Modification enzyme EcoR124I [2,3] using a Magnetic Tweezer system [4,5] to measure the pulling force (in pN) on DNA by the DNA-bound enzyme via motion of a paramagnetic bead attached to one end of the tethered DNA substrate. The original concept for a new device was an electronic version of this Magnetic Tweezer setup, which would be attached to a microfluidics system and allow real time detection of molecular motor activity in a highly parallel, semi-automatic system (Fig. 1). This system could then be used to study a variety of DNA modifying enzymes, many of which are potential targets for drug development (e.g. helicases, topoisomerases and recombinases).

In addition, the project required two other components to be developed, which were seen as a key aspect of developing a Synthetic Biology Project:

1. Construction of cassette-based gene expression systems that would allow over-production of the required proteins and easy purification of these proteins for use in the device. This could act as one part of a documented "biopart" system, which would be an example for other systems.

* Corresponding author. Fax: +44 2392 842070.
E-mail addresses: keith.firman@typei-rm.info (K. Firman), jim.youell@port.ac.uk (J. Youell).

[1] http://royalsociety.org/uploadedFiles/Royal_Society_Content/Events/Summer_Science/2007/Exh19_Biobricks.pdf

[2] https://www.atg-biosynthetics.com/index.php?page=acembl-mix-and-match

0014-5793 © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. Open access under CC BY-NC-ND license.
doi:10.1016/j.febslet.2012.01.057

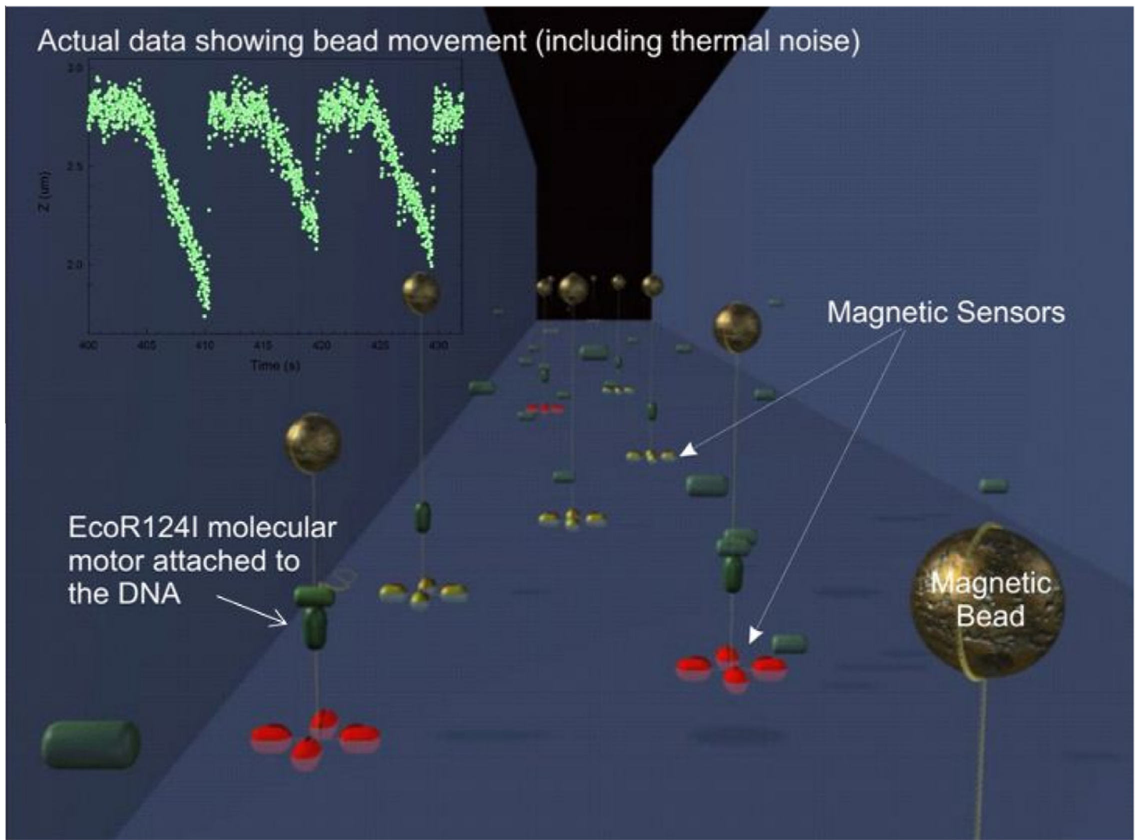

Fig. 1. An electronic Magnetic Tweezer setup in a microfluidics channel. In this initial version of the proposed device Hall Effect sensors [48] were proposed as electronic detectors of vertical bead movement. DNA strands are attached as single molecules above single Hall Effect sensors placed within a microfluidics channel. The DNA with digoxigenin incorporated at one end is surface attached through a dithiobis(succinimidyl propionate)(DSP) to link anti-digoxigenin antibodies on a 100 nm^2 gold patch, which was located above the sensor, while the paramagnetic bead is attached to the DNA though a biotin-streptavidin linkage. An external magnetic field (not shown) holds the attached paramagnetic beads in a vertical position slightly stretching the DNA. The EcoR124I molecular motors (shown in green) are introduced in two stages – the darker green DNA-binding MTase followed by the light green motor component, which attaches to DNA adjacent to the enzyme [49] and translocates the DNA through the bound complex producing supercoiled DNA [50]. This translocation results in vertical movement of the DNA-bound bead, which generates an electrical signal in the Hall Effect sensor (represented by the red colouring) and this output, from a single motor, can be measured in the device and displayed as shown. Random release of the DNA results in resetting of the vertical position of the paramagnetic bead.

2. Construction of DNA substrates. This would have three components to allow surface attachment, bead attachment of the DNA and a central, motor-specific binding site that would ensure directed assembly of the motor at the required site. This can be seen as a variant of the "biopart" system where DNA alone is the "biopart". This is a key aspect of analysing DNA modifying enzymes where different enzymes will need different substrates within the device, but a common method of attachment to surface and bead.

Finally, although initial development was to be based around the EcoR124I molecular motor, a novel system was required to identify problems associated with actual use of the proposed device and to enable development of a potential commercial application. As mentioned above, some types of DNA manipulating enzymes are ATP motors and are consequently useful models for study. DNA helicases were already the subject of studies using Magnetic Tweezer systems [6,7] and seemed a useful source of material. However, we also became particularly interested in one source of novel helicases – *Plasmodium falciparum* – as it was proposed that these enzymes might be used as targets for anti-malarial drug discovery [8]. This would also ensure that substrate development would be a key aspect of the project – designing a variety of special substrates to identify the type of helicase being studied. A great number of studies have been conducted on the bulk assay of helicase activity and it was imperative that a range of substrates reflecting the diverse topologies of DNA *in vivo* were analysed. As well as novel substrates, a range of substrates including synthetic junctions, flaps, bubbles and forks were designed (Fig. 2) as previously described [9,10] to determine the topological preference, polarity, processivity and nucleic acid preference of the helicase motors studied.

Therefore, a clear Synthetic Biology Project was defined and included the following stages:

1. Production of a cassette gene expression system for reliable isolation of molecular motor proteins.
   This would also allow us to determine reliability of the "engineering" approach to gene expression and to detail problems associated with any specific protein production (we believe such information must be an important part of any "bioparts" database).
2. Production of a variety of substrate DNA molecules and assembly of these into a Magnetic Tweezer system.
   Allowing us to better understand how the Plasmodium helicases function and which substrates might be the most appropriate for use in the proposed project.
3. Development of an electronic Magnetic Tweezer setup, including an external magnetic field for lifting the paramagnetic beads and a microfluidic system for delivery of motor proteins to the

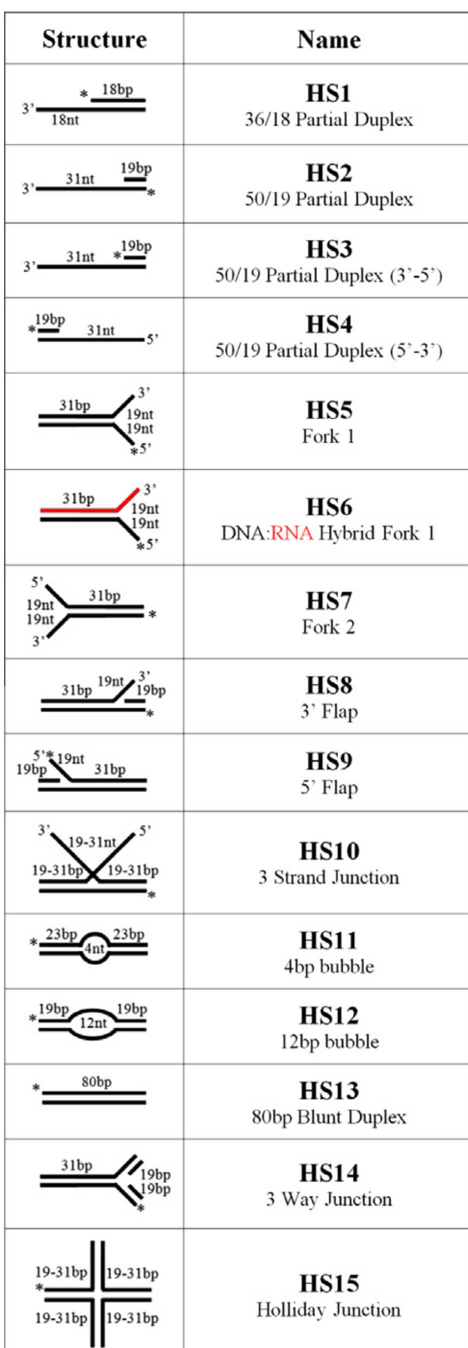

Fig. 2. DNA substrates for analysis of helicase activity. A range of substrates based on those previously described [9,10] were annealed. Visualisation of substrates and unwound products was enabled by the addition of a fluorescein (denoted by *) at the 5′ end of one of the constituent DNA strands.

attached substrate DNA.
This would allow us to gain an understanding of problems associated with substrate attachment above the sensors and the sensitivity of such a device.

4. Investigation of the effect of simple drugs on the function of the Plasmodium helicases.
This would provide the proof of principle for the use of the device in a genuinely commercial situation.

The advantages of using a single molecule sensor for the analysis of drug-target interactions has been clearly demonstrated for Topoisomerase I [11], but another aspect of this project is that developing a nanotechnology-based device could open up the possibility of studying drug–target interactions at the very limit of sensitivity – at or just below the Kd for the interaction.

2. Results and Discussion

The first stage for developing the proposed device was to demonstrate single molecule activity of a DNA-manipulating molecular motor within a conventional, optical Magnetic Tweezer system so that this knowledge could be used in the design and study of the combined optical/electronic Magnetic Tweezer device that was to be developed during this project. The Type I Restriction-Modification enzyme EcoR124I and the DNA translocating enzyme FtsK were the motors used and both enzymes were extensively studied and characterised [2,3,12,13]. The motor subunit of the EcoR124I enzyme was over-produced using a cassette-based system, which included an N-terminal Glutathione *S*-Transferase (GST) tag linked to the *hsdR* gene through a cleavable Prescission™ Protease site [14] and a pET vector based expression system. However, using a His-tag with this protein, at the N-terminus, was found to produce a product with low solubility – an interesting example of the oddities associated with protein production. The former resulted in large amounts of soluble protein, which was easy to purify using the GST tag and allowed assembly of the intact enzyme by mixing with the core DNA Methyltransferase [15]. This work allowed us to develop a simple initial device (without a flowcell) that combined optical and electronic measurement of the magnetic bead (see later).

The first step toward production of a helicase protein for use with the proposed device was to produce significant levels of the PfeIF4A protein from *P. falciparum*. This homologue to the archetypical human DEAD-box helicase, human eukaryotic initiation factor 4A isoform 1 (eIF4A1), is essential for translation initiation and acts as part of a larger initiation factor complex [17]. Comparison of the primary amino acid sequence of PfeIF4A against the human eIF4A (eIF4A1, NCBI accession number NP_001407) showed that the two sequences have identity of 67%, with almost complete conservation of the core helicase superfamily 2 (SF2) motifs. However, the two proteins exhibit significantly different biochemical characteristics and it was hoped that these differences could be exploited in order to develop anti-malarial drugs against this novel target [8,18–20].

2.1. Production and characterisation of helicases from Plasmodium

Analysis of putative helicase genes derived from the genome sequence of *P. falciparum*, identified a total of 45 full-length open-reading frames (ORFs) encoding potential helicases [18,19]. However, helicase proteins isolated in these studies [21–26] were only obtained in low concentrations, which made characterisation difficult. Therefore, we used a simple affinity tag-based expression system for protein production and to ensure high-level production of the proteins in *Escherichia coli* we optimised the codon usage. The full-length open reading frame (NCBI accession number XM_001348793) from *P. falciparum* was synthesised and cloned into pET28a and pGEX6P-1 (a service provided by GeneArt®, Life Technologies Corporation), to yield the recombinants, pJY-GST-PfeIF4A and pJY-His-PfeI4A respectively. Both plasmid constructs were confirmed by restriction fragment mapping and DNA sequencing. Following the model cassette-based system developed for HsdR production, the pJY-GST-PfeIF4A construct was designed to express a fusion protein with a Prescission™ Protease cleavable N-terminal GST tag, whereas the pJY-His-PfeIF4A construct encoded a fusion protein with a thrombin cleavable N-terminal His-tag and a T7 tag [27,28].

Both clones were transformed into E. coli BL21(DE3) [pLysS] for expression of the recombinant gene. A variety of expression tests

using the pJY-GST-PfeIF4A construct demonstrated overproduction of large amounts of protein of the correct molecular weight as insoluble inclusion bodies, and despite various refolding procedures it was not possible to obtain active protein using this construct (another example of the problems of a simple engineering approach to protein production). Work continued on the pJY-His-PfeI4A construct and this yielded smaller amounts of soluble protein after the growth conditions were optimised for maximal production. Therefore, this project has already demonstrated two unexpected situations where a simple "bioparts" approach to protein production was unsuccessful in an unpredictable way.

Pure PfeIF4A was obtained using an extended purification to that described previously [22] in order to remove a contaminating native E. coli helicase (as confirmed by mass spectrometry). In short, a Ni–NTA agarose column (Qiagen) was used to isolate the His-tagged protein. Then this sample was applied to a HiTrap Heparin HP column (GE Healthcare) and subsequently to a HiLoad 26/60 Superdex S200 Prep Grad column (GE Healthcare). Pure samples of PfeIF4A (as determined by SDS–PAGE) were obtained at a final concentration 50 μM and stored as aliquots at -20̊C. The aforementioned presence of an E. coli contaminating helicase was another unexpected observation, which shows that biological systems are not easy to deal with in a simple "engineering-inspired" way! The contaminating protein appeared to bind the PfeIF4A protein through non-covalent association and was co-purified following affinity chromatography.

Following isolation of purified PfeIF4A protein it was necessary to identify which substrate DNA would be the most appropriate for use in a Magnetic Tweezer system. As mentioned previously, a series of fluorescently labelled substrates were adapted from those previously published [9,10] in order to determine the biochemical parameters of the PfeIF4A (Fig. 2). These included branched, nicked and hairpin substrates amongst others. The synthesised fluorescein-tagged substrates allowed the characterisation of the helicase activity using the well documented strand displacement assay that allows the displacement of the annealed strands to be monitored by following the relative gel shift by native gel electrophoresis.

Results from substrate unwinding assays confirmed that PfeIF4A was a slowly processive helicase requiring a substrate with a single stranded region, in order to bind and subsequently unwind substrates, and that the enzyme had significant bipolar activity (unwinding was observed in both 5′-3′ and 3′-5′ directions). Subsequently a Forked substrate (Fork 1 – Fig. 2) was determined as the best substrate to continue with for future Magnetic Tweezer experiments. For Magnetic Tweezer work, the use of various substrates would allow us to determine potential activity on a range of *in vivo* mimics.

However, initials studies of this system using a conventional Magnetic Tweezer system confirmed problems associated with the kinetics of this slow helicase (unpublished observations). Based on the above fluorescent gel based assay, the PfeIF4A helicase exhibits a slow rate of unwinding/translocation, which prevents reliable assays within a Magnetic Tweezer setup. Therefore, at the time of publication of this article, work continues to determine if it will be possible to use single molecule measurement of helicase activity with other Plasmodium-derived proteins, which have been isolated.

2.2. Developing an electronic version of a Magnetic Tweezer setup

It was clear at the outset of this project that two sensing systems were possible for use within the proposed electronic Magnetic Tweezer device:

1. A Hall Effect sensor that might be assembled into a microfluidics channel [29].
   The Hall Effect is the production of a voltage difference (the Hall voltage) across an electrical conductor, transverse to an electric current in the conductor and a magnetic field perpendicular to the current [30], which has been widely used in biosensing systems involving detection of changing magnetic fields [31,32].
   This approach was an interesting exploration of a novel technique for assembling the Hall Effect sensors using an electron beam to generate the conductors [33], which would be an important contribution to basic science and, hopefully, to the project.
2. A Magnetoresistive sensor [34], which could be readily constructed within a microfluidics environment.
   The magnetoresistive effect is a quantum mechanical effect, due to electron spin, in which a significant reduction of electrical resistance is produced in a thin-layer structure of alternating magnetic and non-magnetic layers in the presence of an external magnetic field [35]. Our collaborators had developed this technology for detection of DNA on a chip [36], which was a useful place to start this project.
   The hope with this approach was for the rapid development of a chip-based device as there was a significant amount of knowledge regarding the use of these devices with biological systems [34,36–39].

Much of the initial work with the Hall Effect sensors revolved around reliability and sensitivity measurements as the construction technique was novel and this work included preparation of nano-sized paramagnetic particles for detection at the limits of sensitivity using the prepared Hall Effect sensors and more conventional versions of Hall Effect sensors. This work is on going and it has become clear that the concept is possible, but we have yet to realise construction of a useful device.

Progress with the magnetoresistive device was both rapid and successful. The first stage development was based on a hanging drop system (Fig. 3A) where magnetic beads and biomolecules were introduced in a 20 μl drop of buffered solution. However, a major problem encountered with this system was attachment of the biomolecules above the sensor. Initial attempts to attach DNA to the surface above the sensor were based on a chemical attachment using a thiol group incorporated onto the DNA substrate and a gold-coated surface, located above the magnetoresistive sensor. However, this technology gave poor efficiency of binding and resulted in non-specific interactions within the system, as well as making regeneration of the chips difficult. Improved surface attachment techniques were developed using dithiobis(succinimidyl propionate)(DSP) to link anti-digoxigenin antibodies to a $100nm^2$ gold patch, which was located above the sensor.

The DNA substrates were, therefore, designed from three DNA products (Fig. 4) with relevant restriction sites to allow easy directional ligation: a short PCR product incorporating digoxigenin-11-UTP (for binding to the antibody functionalised gold surface), the DNA substrate of interest, and another short PCR product incorporating biotin-16-dUTP (for binding to a streptavidin functionalised paramagnetic bead). The inclusion of multiple modified nucleotides at either end of the substrate were hoped to crowd the relevant surface to be attached and push the ratios of binding to 1:1 in each case, which was confirmed using Scanning Electron Microscopy (SEM) based analysis of functionalised/DNA bound/washed surfaces. In addition, this initial device incorporated a glass base, which allowed observation of the bead in a standard optical Magnetic Tweezer system and consequently dual measurement of bead movement by optical and electronic means. Development of the initial drop-based MRMT device was described by Chaves et al. [40].

Following this initial development of a single drop device, which was used to confirm detection of, movement of the paramagnetic bead and attachments of the DNA substrates for use with the EcoR124I motor, a device was proposed, incorporating

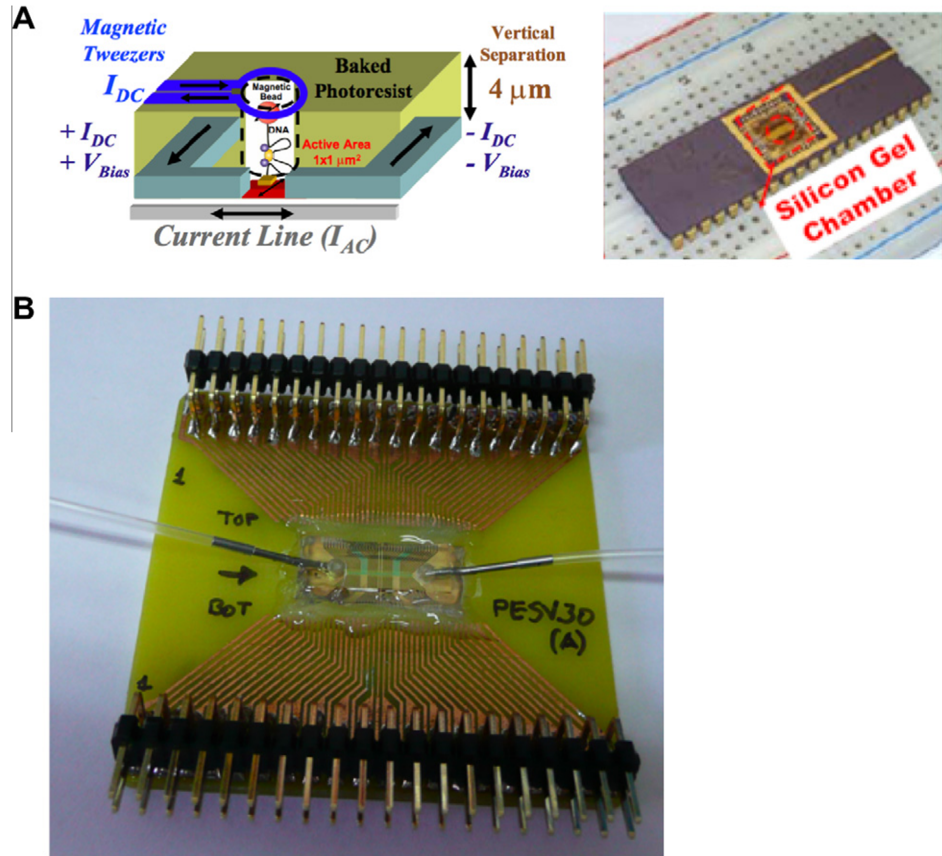

Fig. 3. A prototype MagnetoResistive Magnetic Tweezer (MRMT) system. (A) On the left is a cartoon representation of the first prototype MRMT device, which is illustrated as a breadboard device on the right side of the figure. The required external magnetic field, required to hold up the paramagnetic bead and stretch the attached DNA, is provided by a small coil (in blue) powered by I_{DC} while the magnetoresistive sensor is illustrated in grey [40]. (B) A simple microfluidics flowcell was produced with multiple sensors as in (A) and connected to external input using a simple connector [40].

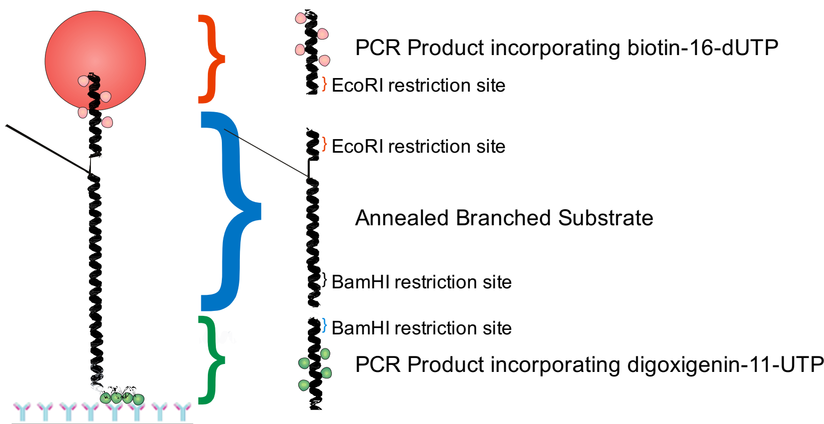

Fig. 4. Method of attachment of DNA substrates in a Magnetic Tweezer setup. Example of a composite fork-like substrate attached to a paramagnetic bead and a functionalised gold surface. Binding of the substrate to the bead is mediated through the interaction of multiple biotin molecules and the streptavidin functionalised surface of the bead. Binding to the surface is via a specific interaction with anti-DIG antibodies, which in turn interact with the surface via the DSP cross-linker. The substrate DNA, highlighted by the blue bracket – } – can be any of the substrates illustrated in Fig. 2.

a simple flowcell attached to the MagnetoResistive Magnetic Tweezer (MRMT) device, to deliver biomaterials to the sensor. Finally, the DC operated coil used to hold up the paramagnetic bead also enables vertical manipulation of the bead during surface attachment of the DNA, which overcomes non-specific attachment of the bead within the chamber. Following this initial work the proposed simple flowcell version of the device was constructed with multiple MT areas within the flow-chamber and attached to a feeder inlet of 100 μl volume (Fig. 3B).

While the real potential of this MRMT device has yet to be demonstrated, some of the capabilities include:

1. The use of pulsed electrical current in the coil to wind DNA so as to create supercoils in the DNA for use with some substrates (e.g. topoisomerases).
2. The potential to measure with smaller beads that cannot be observed using an optical system, which would reduce the effect of the bead on motor activity.

3. The possibility of parallel channels that allow direct comparison of the same motor/substrate combinations in the presence of different temperature, flow rates and in the presence or absence of drugs and other chemicals.
4. Development of a simple on/off measurement of a drug affecting motor activity for rapid screening of drugs against targets.
5. The potential for measurement of drug–target interactions at very low drug concentrations.
6. Development for use with novel DNA substrates (e.g. quadruplex DNA [41]) and the folding of aptamers for biosensing [42,43].
7. Development of a system for the study of protein–protein interactions, based around the model twin-hybrid system, where the DNA substrate in the Magnetic Tweezer is looped by the interaction between two proteins fused to two different DNA-binding proteins.

2.3. Design of single molecule experiments to detect Plasmodium helicase activity

Design of substrates for either the optical (conventional) Magnetic Tweezer or the electronic MMT device required the use of modified versions of the bulk assay substrates in order to allow the correct alignment of substrates with the surface. For either device the choice of microfluidic surface attachment was through the specific interaction of digoxigenin(DIG)-11-UTP with anti-DIG antibodies bound to the surface. Binding with the paramagnetic bead was reliant on the well documented streptavidin–biotin interaction and therefore there was a need to incorporate a region of biotin-16-dUTP in a region of the DNA substrate. Substrates were made by ligating components (using a similar method to that described earlier for the EcoR124I substrates [2] an example of which is shown in Fig. 4) and annealing of complimentary strands to produce the desired substrate to mimic those described previously.

With a range of substrates developed as described it is easy to see how a high throughput translocase/helicase characterisation and drug screening system could be produced. With a correctly implemented multi channel microfluidic system with multiple sensing devices in each, the analysis of enzyme activity and also drug inhibition, on different substrates, could be monitored in parallel with repeats on the same chip. Alternatively, simply by using the same substrate throughout the system, libraries of inhibitors could be screened for specific hits against one target enzyme quickly and effectively.

3. Conclusions

In this paper we describe an ambitious Synthetic Biology and Bionanotechnology Project, which has involved three consecutive research grants funded by European sources. The concept was to construct an electronic device for single-molecule measurement of drug–target interactions at the very limit of sensitivity. The project is still ongoing at the time of writing this review, but significant progress has been made from the first steps, which involved answering the question "*can we detect movement of a magnetic bead attached to DNA, using a single molecule molecular motor to manipulate the DNA*"?

We have documented the capability of an optical Magnetic Tweezer system for measurement of movement by two DNA translocators (EcoR124I [2] and FtsK [12]) and fully characterised DNA translocation by these molecular motors. This work was then expanded to characterise other motors, including DNA helicases [7], which led to the concept of expanding this study to the characterisation of novel helicases from *Plasmodium falciparum*, which are potential drug targets for development of new anti-malarial drugs [8]. At this time work continues to identify a suitable helicase for measurement with the MRMT device developed during the project, but the device has many other potential uses that can be developed.

A Synthetic Biology approach for isolation of new recombinant clones of the Plasmodium helicase genes was adopted and proteins purified from *E. coli* strains that guaranteed high-level production of the proteins. The helicases have been characterised using standard techniques and single molecule characterisation has just started at the time of preparation of this paper. However, we have also documented problems associated with this engineering-based approach of using a cassette system to link "bioparts" as well as interesting (unexpected) problems associated with protein purification. Therefore, the Project has provided strong evidence that any database of bioparts must include such negative observations to enable productive use of the bioparts.

Finally, we have successfully constructed an electronic version of a Magnetic Tweezer system, demonstrated assembly of DNA substrates within the device and motor activity [40]. This device is an important example of incorporating a biological system into a micro-engineered construct. Therefore, the project has been very successful and the possibilities now exists of developing a commercial device that can detect drug–target interactions at the single molecule level, where sensitivity should be based on the kd for the interaction, and characterise the nature of these interactions.

The future for this project could be wide ranging, with the possibility of using the device for the study of systems that involve changes to DNA topology – aptamers [44], where the device could be used as a biosensor for detection of the aptamer target molecule; quadruplex DNA [45], where DNA-binding drugs that inhibit quadruplex formation might be identified and used for specific disease treatments [46]; the study of protein-protein interactions by adapting the system to mimic the DNA looping concept of the two-hybrid system [47], etc.

This project has shown that applying engineering techniques to biological systems can work very well and that incorporation of nanotechnology and miniaturisation will produce novel devices with a wide ranging capability for the future.

Acknowledgements

The early studies of the molecular motor activity of EcoR124I were supported by EC funding through the FET-OPEN scheme (MOLSWITCH, Grant no. IST-2001-38036).

The development of an electronic Magnetic Tweezer device was supported by EC funding through the NEST scheme (BIONANO-SWITCH, Grant no. 043288).

Current work investigating the potential for measuring single-molecule drug–target interactions was supported through funding from EPSRC under the EC-supported NanoSci-E+ scheme (MOL-MACHINES, Grant no. EP/H006702/1). J.Y. would like to thank EPSRC for this funding, which has provided him with the opportunity to develop this technology with Plasmodium helicases.

Background to the projects and a list of collaborators is available at http://www.bionano-switch.info/

References

[1] Sorensen, H.P. (2010) Towards universal systems for recombinant gene expression. Microb. Cell Factories 9, 27.

[2] Seidel, R. et al. (2004) Real-time observation of DNA translocation by the Type I restriction-modification enzyme EcoR124I. Nat. Struct. Mol. Biol. 11, 838–843.

[3] Seidel, R., Bloom, J.G.P., van Noort, J., Dutta, C.F., Dekker, N.H., Firman, K., Szczelkun, M.D. and Dekker, C. (2005) Dynamics of initiation, termination and reinitiation of DNA translocation by the motor protein EcoR124I. Eur. Mol. Biol. Organ. J. 24, 4188–4197.

[4] Chiou, C.-H., Huang, Y.-Y., Chiang, M.-H., Lee, H.-H. and Lee, G.-B. (2006) New magnetic tweezers for investigation of the mechanical properties of single DNA molecules. Nanotechnology 17, 1217–1224.

[5] Gosse, C. and Croquette, V. (2002) Magnetic Tweezers: micromanipulation and force measurement at the molecular level. Biophys. J. 82, 3314–3329.

[6] Lionnet, T. et al. (2006) DNA mechanics as a tool to probe helicase and translocase activity. Nucleic Acids Res. 34, 4232–4244.

[7] Lionnet, T., Spiering, M.M., Benkovic, S.J., Bensimon, D. and Croquette, V. (2007) Real-time observation of bacteriophage T4 gp41 helicase reveals an unwinding mechanism. Proc. Natl. Acad. Sci. USA 104, 19790–19795.

[8] Tuteja, R. (2007) Helicases; feasible antimalarial drug target for *Plasmodium falciparum*. FEBS J. 274, 4699–4704.

[9] Ozsoy, A.Z., Ragonese, H.M. and Matson, S.W. (2003) Analysis of helicase activity and substrate specificity of Drosophila RECQ5. Nucleic Acids Res. 31, 1554–1564.

[10] Anand, S.P. and Khan, S.A. (2004) Structure-specific DNA binding and bipolar helicase activities of PcrA. Nucleic Acids Res. 32, 3190–3197.

[11] Koster, D.A., Palle, K., Bot, E.S.M., Bjornsti, M.-A. and Dekker, N.H. (2007) Antitumour drugs impede DNA uncoiling by topoisomerase I. Nature 448, 213–217.

[12] Saleh, O.A., Perals, C., Barre, F.X. and Allemand, J.F. (2004) Fast, DNA-sequence independent translocation by FtsK in a single-molecule experiment. Eur. Mol. Biol. Organ. J. 23, 2430–2439.

[13] Saleh, O.A., Bigot, S., Barre, F.X. and Allemand, J.F. (2005) Analysis of DNA supercoil induction by FtsK indicates translocation without groove-tracking. Nat. Struct. Mol. Biol. 12, 436–440.

[14] Libby, R.T., Cosman, D., Cooney, M.K., Merriam, J.E., March, C.J. and Hopp, T.P. (1988) Human rhinovirus 3C protease: cloning and expression of an active form in *Escherichia coli*. Biochemistry 27, 6262–6268.

[15] Patel, J., Taylor, I., Dutta, C.F., Kneale, G.G. and Firman, K. (1992) High-level expression of the cloned genes encoding the subunits of and the intact DNA methyltransferase, M.*Eco*R124. Gene 112, 21–27.

[17] Chang, J.H., Cho, Y.H., Sohn, S.Y., Choi, J.M., Kim, A., Kim, Y.C., Jang, S.K. and Cho, Y. (2009) Crystal structure of the eIF4A-PDCD4 complex. Proc. Natl. Acad. Sci. USA 106, 3148–3153.

[18] Tuteja, R. and Pradhan, A. (2006) Unraveling the 'DEAD-box' helicases of *Plasmodium falciparum*. Gene 376, 1–12.

[19] Tuteja, R. (2010) Genome wide identification of *Plasmodium falciparum* helicases: a comparison with human host. Cell Cycle 9, 104–120.

[20] Tuteja, R. and Pradhan, A. (2010) PfeIF4E and PfeIF4A colocalize and their double-stranded RNA inhibits *Plasmodium falciparum* proliferation. Commun. Integr. Biol. 3, 611–613.

[21] Pradhan, A., Chauhan, V.S. and Tuteja, R. (2005) A novel 'DEAD-box' DNA helicase from *Plasmodium falciparum* is homologous to p68. Mol. Biochem. Parasitol. 140, 55–60.

[22] Pradhan, A. and Tuteja, R. (2007) Bipolar, dual *Plasmodium falciparum* helicase 45 expressed in the intraerythrocytic developmental cycle is required for parasite growth. J. Mol. Biol. 373, 268–281.

[23] Shankar, J., Pradhan, A. and Tuteja, R. (2008) Isolation and characterization of *Plasmodium falciparum* UAP56 homolog: evidence for the coupling of RNA binding and splicing activity by site-directed mutations. Arch. Biochem. Biophys. 478, 143–153.

[24] Suntornthiticharoen, P., Petmitr, S. and Chavalitshewinkoon-Petmitr, P. (2006) Purification and characterization of a novel 3′-5′ DNA helicase from *Plasmodium falciparum* and its sensitivity to anthracycline antibiotics. Parasitology 133, 389–398.

[25] Prakash, K. and Tuteja, R. (2010) A novel DEAD box helicase Has1p from *Plasmodium falciparum*: N-terminal is essential for activity. Parasitol. Int. 59, 271–277.

[26] Shankar, J. and Tuteja, R. (2008) UvrD helicase of *Plasmodium falciparum*. Gene 410, 223–233.

[27] Studier, F.W. and Moffatt, B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. 189, 113–130.

[28] Smith, D.B. and Johnson, K.S. (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene 67, 31–40.

[29] Boero, G. et al. (2005) Submicrometer Hall devices fabricated by focused electron-beam-induced deposition. Appl. Phys. Lett. 86, 042503 (online).

[30] Leong, Z.Y., Tan, S.G., Jalil, M.B.A., Kumar, S.B. and Han, G.C. (2007) Magnetoresistance modulation due to interfacial conductance of current perpendicular-to-plane spin valves. J. Magn. Magn. Mater. 310, e635–e637.

[31] Moritz, J., Rodmacq, B., Auffret, S. and Dieny, B. (2008) Extraordinary Hall effect in thin magnetic films and its potential for sensors, memories and magnetic logic applications. J. Phys. D Appl. Phys. 41, 135001.

[32] Sandhu, A., Kumagai, Y., Lapicki, A., Sakamoto, S., Abe, M. and Handa, H. (2007) High efficiency Hall effect micro-biosensor platform for detection of magnetically labeled biomolecules. Biosens. Bioelectron. 22, 2115–2120.

[33] Utke, I., Hoffmann, P., Berger, R. and Scandella, L. (2002) High-resolution magnetic Co supertips grown by a focused electron beam. Appl. Phys. Lett. 80, 4792–4794.

[34] Graham, D.L., Ferreira, H.A. and Freitas, P.P. (2004) Magnetoresistive-based biosensors and biochips. Trends Biotechnol. 22, 455–462.

[35] Hinchey, L.L. and Mills, D.L. (1986) Magnetic properties of superlattices formed from ferromagnetic and antiferromagnetic materials. Phys. Rev. B 33, 3329–3343.

[36] Freitas, P.P., Ferreira, H.A., Graham, H.L., Clarke, L.A., Amaral, M.D., Martins, V., Fonseca, L. and Cabral, J.S. (2004) Magnetoresistive DNA chips in: Magnetoelectronics (Johnson, M., Ed.), pp. 331–374, Elsevier Academic Press, New York.

[37] Graham, D.L., Ferreira, H.A., Freitas, P.P. and Cabral, J.M. (2003) High sensitivity detection of molecular recognition using magnetically labelled biomolecules and magnetoresistive sensors. Biosens. Bioelectron. 18, 483–488.

[38] Schotter, J., Kamp, P.B., Becker, A., Puhler, A., Reiss, G. and Bruckl, H. (2004) Comparison of a prototype magnetoresistive biosensor to standard fluorescent DNA detection. Biosens. Bioelectron. 19, 1149–1156.

[39] Wirix-Speetjens, R., Reekmans, G., De Palma, R., Liu, C., Laureyn, W. and Borghs, G. (2007) Magnetoresistive biosensors based on active guiding of magnetic particles towards the sensing zone. Sens. Actuators, B 128, 1–4.

[40] Chaves, R.C., Bensimon, D. and Freitas, P.P. (2011) Single molecule actuation and detection on a lab-on-a-chip magnetoresistive platform. J. Appl. Phys. 109.

[41] Gómez-Márquez, J. (2010) DNA G-quadruplex: structure, function and human disease. FEBS J. 277, 064702-1–064702-8.

[42] Achenbach, J.C., Nutiu, R. and Li, Y. (2005) Structure-switching allosteric deoxyribozymes. Anal. Chim. Acta 534, 41–51.

[43] Balamurugan, S., Obubuafo, A., Soper, S. and Spivak, D. (2008) Surface immobilization methods for aptamer diagnostic applications. Anal. Bioanal. Chem. 390, 1009–1021.

[44] Lin, C.H. and Patel, D.J. (1997) Structural basis of DNA folding and recognition in an AMP-DNA aptamer complex: distinct architectures but common recognition motifs for DNA and RNA aptamers complexed to AMP. Chem. Biol. 4, 817–832.

[45] Gray, R.D. and Chaires, J.B. (2011) Linkage of cation binding and folding in human telomeric quadruplex DNA. Biophys. Chem. 159, 205–209.

[46] Wu, Y. and Brosh, R.M. (2010) G-quadruplex nucleic acids and human disease. FEBS J. 277, 3470–3488.

[47] Luban, J. and Goff, S.P. (1995) The yeast two-hybrid system for studying protein–protein interactions. Curr. Opin. Biotechnol. 6, 59–64.

[48] Middelhoek, M.G. (2004) Hall effect devices. Sens. Actuators, A 116, 185.

[49] van Noort, J., van der Heijden, T., Dutta, C.F., Firman, K. and Dekker, C. (2004) Initiation of translocation by type I restriction-modification enzymes is associated with a short DNA extrusion. Nucleic Acids Res. 32, 6540–6547.

[50] Szczelkun, M.D., Dillingham, M.S., Janscák, P., Firman, K. and Halford, S.E. (1996) Repercussions of DNA tracking by the type IC restriction endonuclease *Eco*R124I on linear, circular and catenated substrates. Eur. Mol. Biol. Organ. J. 15, 6335–6347.