

# MOL SWITCH

## Monthly Newsletter

July 2002  
Year 1, Issue 1

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Mol Switch Coordinator  
University of Portsmouth  
King Henry Building, Portsmouth  
PO1 2DY, UK

### Reason for monthly Newsletter

*Keith Firman*

The idea arose for this newsletter from discussions with another Consortium Coordinator based here in Portsmouth. They found that the amount of email correspondence between Consortium Members was so great that there was a need to summarise the main points for everyone and therefore keep everyone fully informed.

This is the first issue and I plan to use this to set the scene for the work we are about to embark upon.

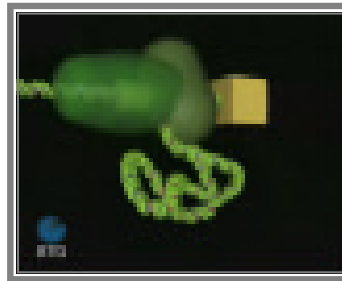
#### **Current state of play with MOL SWITCH**

We managed to meet the deadline with our submission of the contract preparation forms and since then I have forwarded the signed hard-copies to Ramon Compano.

I have received a letter back from the Commission office indicating that I will shortly receive a PDF-file version of the actual contract, which we should scrutinise and then sign two copies, which need to be returned to me. I have faxed everyone a copy of this letter so that you fully understand what the EC expects.

As soon as I receive the PDF file I will forward this to everyone.

Once you have all sent the **two**, signed hard-copies of the contract to me I will forward these copies, with my own, to the EC. The commission will then sign them and we start the grant the first day of the month following this signature. Currently this is believed to be October 1<sup>st</sup> 2002.



### Coordinator's Administrator

*Keith Firman*

To assist me with this project I have claimed sufficient funds to pay a one-quarter salary for an administrator post. As it is extremely unlikely that anyone else would work for such a low income my wife has agreed to 'do the job'.

I know many of you have met Kathy (McMahon – she uses her previous surname for legal reasons) – and are aware that she is a very good organiser. One of her main tasks will be to arrange visits and project workshops and I am sure she will be excellent at this job (the food and accommodation is usually good when Kathy is involved).

*[Continued on page4...](#)*

# Fluorescence work (Workpackage 7)

Roberto Favilla

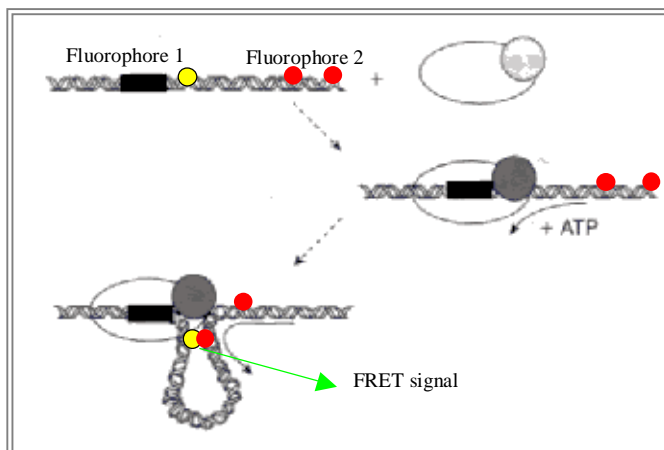
Concerning the project, I have been thinking a little bit about (*the work*) in these last weeks, in order to find good strategies to detect FRET signals from motor-DNA complexes. This is also important to (*help*) decide which instrumental setup is most appropriate to be purchased. It seems to me that at least for the first 1 or 2 years of the project it would perhaps better to try and work on freely diffusing molecules in solution, though we have the possibility

to perform AFM (fluorescence?) measurements on molecules deposited on a surface. The approach in solution has to face DNA coiling. I have no clear ideas on how big this problem could be.

One possibility to bypass, at least in

part, this problem could be to work with relatively short molecules, of the order of the persistence length of dsDNA, which, if I am not wrong, should be of the order of 100-200bp. If instead this is not a problem, DNA molecules of any length could in principle be used. I think

however that problems will arise with increasing lengths, because, even assuming that (*the*) start of DNA movement can be perfectly synchronized, e.g. by flash photolysis using caged ATP, stochastic events will take over soon or later, with loss of synchronism. How large this effect will be is however to be checked experimentally.



For FRET experiments, it would be very useful to

decide the nature of the donor-acceptor molecules to be used. A good couple seems to be TRM (tetramethylrhodamine) as donor and Cy5 as acceptor. This is important to be established at the beginning (*of the project*) because the laser to be used for excitation depends on it. Simultaneous (2-channel) detection of the donor and acceptor FLU signals, should provide a way to detect FRET of single molecules passing into the confocal laser-excited micro-volume, timing should be given by the flash photolysis event. Accumulating a sufficient number of events should provide the necessary statistics to establish the most probable time of a given FRET couple. The possibility to sequence DNA will mostly depend on how accurate timing will be. Selective labelling of each base pair should increase the accuracy of the analysis, because this would reduce signal broadening with loss of synchronized movement. However, in principle, each base should have a most probable FRET timing. If one can correlate the timing of each FRET signal with the distance of each bp from

## Consortium Details

This section will hopefully grow as the new Consortium Members are appointed. Please email further details of group members to: [mcmahonka@ntlworld.com](mailto:mcmahonka@ntlworld.com).

### Portsmouth University, United Kingdom (Ports)

**Corodinator** – Keith Firman ([keith.firman@port.ac.uk](mailto:keith.firman@port.ac.uk)).  
**Administrator** – Kathy McMahon. ([mcmahonka@ntlworld.com](mailto:mcmahonka@ntlworld.com))

**Molecular Motor Group Members** – Sheelagh Campbell (silicon etching, surface chemistry and AFM), Christina Dutta (protein and DNA preparations), David Franklin (magnetics), Darren Mernagh (DNA-binding studies, motor function and fluorescence techniques) & James Smith (AFM), A.N.Other. to be appointed.

### National Physical Laboratory, United Kingdom (NPLML)

**Project Leader** – John Gallop ([john.gallop@npl.co.uk](mailto:john.gallop@npl.co.uk)).

### Technology University Delft, The Netherlands (TUDelft)

**Project Leader** – Cees Dekker ([dekker@qt.tn.tudelft.nl](mailto:dekker@qt.tn.tudelft.nl)).

### CNRS, Ecole Normale Supérieure, France (CNRS/ENS)

**Project Leader** – David Bensimon ([david@lps.ens.fr](mailto:david@lps.ens.fr))

### Universita' di Parma, Italy (Parma)

**Project Leader** – Roberto Favilla ([roberto.favilla@fis.unipr.it](mailto:roberto.favilla@fis.unipr.it))

### Institute of Microbiology, Czech Academy of Sciences (IMIC)

**Project Leader** – Marie Weiserova ([weisero@biomed.cas.cz](mailto:weisero@biomed.cas.cz)).

a reference position, sequencing should be possible. All this requires a confocal microscope excited with an argon ion laser, to excite a volume of about 1 micron<sup>3</sup>, so that no more than one molecule of a 1 nM solution on average will pass through it, plus sophisticated APD detectors for single molecule signals, plus a flash photolysis system to trigger ATP activation, to be synchronised with data acquisition, etc. etc.

These ideas are a preliminary attempt to rationalize our future work. I would be pleased to receive comments as well as inputs from you too.

### Comments from KF and DM

*We agree that the use of short molecules may be advantageous. Preliminary studies using the AFM facilities at TUDelft show a 600bp fragment can form loops following translocation, but doesn't seem to coil too much. The question that arises is how short can the molecule be for us still to see translocation (the DNA will need to be long enough to separate the two fluorophores to prevent FRET until the motor moves the DNA – assuming one fluorophore is on the motor (at least during initial experiments) and one on the DNA.*

Feedback to [keith.firman@port.ac.uk](mailto:keith.firman@port.ac.uk)

## Proposed Meeting of Consortium

Keith Firman

A number of people in the Consortium have suggested that an early meeting to discuss everyone's role, and to outline lines of communication, would be useful. There is also a need for transfer of materials soon after day zero (e.g. most groups will need motor protein).



One of the key roles for Portsmouth is to supply protein and DNA material and this suggests the inaugural meeting should be in Portsmouth. I feel that a Friday would be the best day allowing people time to travel on Thursday and return over the weekend, or when they feel it is most appropriate (freezer storage of materials is possible over this weekend).

So we propose to meet in Portsmouth on **4<sup>th</sup> October 2002**.

Feedback on this meeting to [mcmahonka@ntlworld.com](mailto:mcmahonka@ntlworld.com).

Where we (Ports) need to provide materials for people we will also provide insulating ice boxes filled with ice-blocks to allow transportation of the material. However, please be aware that this is just the beginning of the project for us as well we may have both limited stocks of protein and only a limited number of substrate molecules.

## Day Zero - The Workpackages

Keith Firman

So what are the various groups expected to do on day zero?

### Workpackage 1

Ports will provide IMIC with the cloned genes for the *hsdR(R124)* and *hsdR(prr)* motor proteins. IMIC will then carry out site-directed mutagenesis at motif X to produce motor subunits that cannot cleave DNA.

### Workpackage 2

Ports will provide the molecular motor protein (core MTase, HsdR(prr) and HsdR(R124) to NPLML, TUDelft, ENS/CNRS and Parma. This

material will be available from day zero. We will also have various 'conventional' DNA substrates such as the plasmid pCFD30 (single site for motor binding, ~3kbp). However, labelled substrates (with fluorophores and biotin/DIG etc) will take time to produce as will short synthetic substrates, but these will be made available to the rest of the Consortium as soon as possible.

Ports will initiate AFM measurements of translocation in collaboration with NPLML.

Ports, TUDelft and ENS/CNRS will initiate magnetic tweezer force measurements with the R<sub>1</sub>-complex.

### Workpackage 3

Ports will produce etched silicon wells and channels for small volume motor activity assays.

Ports will investigate reliable assays for motor activity (e.g. ATPase measurements) and determine the limits for both volume and concentration and the effect of large silicon surface areas on motor activity.

TUDelft, ENS/CNRS and NPLML will initiate studies on surface passivation and how this affects motor activity and/or non-specific DNA binding on surfaces.

### Workpackage 4

Ports will provide palindromic DNA substrates for ENS/CNRS to initiate studies with magnetic tweezers.

### Workpackage 5

Ports and NPLML will initiate magnetic particle production to prepare novel magnetic particles for DNA attachment.

### Workpackage 6

This doesn't start until month 10.

### Workpackage 7

(Also see page 2). Parma will initiate measurements of FRET signals between labelled motor protein and translocated DNA.

## 6<sup>th</sup> month report (Workpackage 0)

The project administrator, appointed by the Project Co-ordinator, will have produced a web-site describing the project and its progress (hosted at the NanoNet Network site: <http://www.nanonet.org.uk>), established a Steering Committee for management of the science and established lines of communication with all Consortium Members and the EU. A summary of the 'state of play' and the scientific progress will be collated by gathering brief summaries from Consortium Members. This will be distributed to the Steering Committee for comment and discussion. This will provide a working arrangement for review of the direction of the research and allow a rapid response to research that is failing

*Continued from Page 1*

Kathy will also provide the primary contact for non-scientific email and the best means of addressing issues related to funding or general administration of the project. Finally, she will edit this Newsletter!

If you want to contact Kathy her email address is [mcmahonka@ntlworld.com](mailto:mcmahonka@ntlworld.com)

to meet expectations.

In addition, we will be required to prepare a detailed description concerning exploitation and control of IP for the remainder of the project. I imagine people's views on this matter will provide a lengthy Newsletter for February 2003.

## Announcement of posts available

*Various*

Following are advertisements for two research posts that are funded from the FET-OPEN grant if you know of any likely candidates please ask them to make contact:

### UNIVERSITY OF PORTSMOUTH

A position exists at Portsmouth University for an experienced Postdoctoral Worker who is interested in the exciting new field of nanotechnology. As a cross-discipline science there will be an opportunity to broaden your knowledge and gain experience through involvement with biologists, chemists and physicists. The EU funds the project and you will join a pan-European Consortium comprising the National Physical Laboratory UK, Technical University, Delft, CNRS Paris, University of Parma, Italy and the Institute of Microbiology, Prague, Czech Republic. Therefore there will be many opportunities to expand your research experience.

### Project Description:

The success of the Human Genome Sequencing Project means that DNA sequence information is one of the greatest stores of information and is now of interest to individuals. This project will initiate work to construct a single-molecule DNA-sequencing

## Initial Events

**1<sup>st</sup> October 2002**

***Proposed start date for project***

This assumes the Commission sign the contracts during September.

**4<sup>th</sup> October 2002**

**11.00, Location to be announced  
*Initial meeting of full consortium***

See across page for further details.

## Key Dates

**1<sup>st</sup> March 2003**

***6<sup>th</sup> month report stage***

In the contract forms you will see I was asked to list the deliverables associated with Coordination of the project at the end of six months See across page for full details.

**September 2005**

**Location to be announced  
*Workshop on "DNA-based approaches to sensing and electronics"***

This was part of the contract negotiations between KF and Ramon Compano. He wants to involve members of the Phantoms Network in this workshop.

device, but closely linked to this development process, we will also construct a nano-switch, based on a biological molecular motor and a moving magnetic bead. This device will provide a link between the biological world and the silicon-based microelectronics world. Such a device is likely to find longer-term uses in the development of a wide range of devices from biosensors to 'new generation' prosthetics and artificial limbs constructed with controlling computer

devices. In addition, the magnetic bead attached to DNA can be used in the single-molecule DNA sequencing apparatus (to overcome coiling due to entropy) and for monitoring the movement of the DNA molecule.

#### Research experience

The candidate should have at least four years experience at postdoctoral level with skills that cross between Chemistry and Molecular Biology and a willingness to travel to the other research laboratories around Europe to exchange data and materials. Ideally, the candidate should have experience in any of the following - the handling of single molecules, atomic force microscopy, chemical modifications of surfaces, or biological molecule-substrate interactions, however, this is not essential and candidates with a strong interest in developing new skills, or an interest in nanotechnology will be considered.

Salary approx. £21,000 dependent upon age and experience.

#### Start date 1<sup>st</sup> October 2002

For further details and an application form contact:

Dr. Keith Firman  
School of Biological Sciences  
University of Portsmouth  
King Henry Building  
King Henry I Street  
Portsmouth PO1 2DY  
United Kingdom  
Tel. (+44) 02392 842059  
Fax (+44) 02392 842070

#### UNIVERSITY OF PARMA

A three-year post-doctoral fellowship, financed by the European Community, will be available from October 2002 at the University of Parma, Italy, on a research project of the Information Society and Technology (IST) Program-V Framework.

The project, entitled "A molecular magnetic switch that links the biological and silicon worlds" (acronym MOL-SWITCH), will deal with development of nanodevices for sequencing DNA at the level of single molecule. We will mainly deal with the exploitation of the fluorescence resonance energy transfer (FRET) effect between a single pair of donor-acceptor molecules. The acceptor will be conjugated to a protein molecular motor, while the acceptor to selected DNA bases. The post-doc fellow will work on this interdisciplinary project, in a biochemical and biophysical environment, using advanced instrumentation, like confocal microscopy for single molecule detection. Therefore, candidates will be selected on the basis of their expertise in fluorescence energy transfer microscopy and single molecule techniques, in addition to biochemical and biophysical laboratory experience. The research activity will be mainly carried out at the University of Parma, Departments of Biochemistry and Molecular Biology and Department of Physics-INFN Unit, Italy. The gross salary will be 25.000 Euro/Year.

Applications, including a full CV, should be sent by e-mail to Prof. Roberto Favilla, Department of Physics, University of Parma, roberto.favilla@fis.unipr.it, using "mol-switch application" as subject, before the end of August 2002. Foreseen start of the project is October 2002.