Biochemical reconstitution and AFM (atomic force microscopy) observation of the protein–DNA complexes involved in DNA replication

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To understand the regulation of DNA-mediated reactions, the structural analysis of the protein–DNA complexes is required, because the most of the enzymatic, cooperative or exclusive activities of the proteins have been explained by the conformation of the protein–DNA complexes. DNA replication is known as the process regulated by the various protein-DNA complexes, but the replication complexes formed on the DNA had rarely been visualized, because these complexes are too large and heterogeneous to be analyzed by X-ray crystallography or cryo-EM.

We applied atomic force microscopy (AFM) to observe protein–DNA complexes involved in DNA replication, because AFM allows us to observe DNA and proteins in the nanometre scale without crystallization or averaging of the images. The reaction of DNA replication is divided into these four steps; (1) the determination of the chromosomal regions called 'replication origins' where DNA replication starts, (2) licensing of the DNA replication (formation of the pre-replicative complex, pre-RC, on origins) at G1 phase, (3) firing of origins (unwinding of dsDNA at origins) at S phase, and (4) elongation of the newly synthesized DNA (replication fork progression). We reconstituted these reactions using purified proteins and DNA, and analyzed it by AFM. We found origin recognition complex (ORC) establishes origin-specific interactions by binding both to origin DNA and to neighboring nucleosomes [1]. We also reconstituted pre-RC and observed a DNA loop fastened by the MCM double hexamer was detected [2]. From the reconstitution of the fork progression and pausing [3], we detected 'branched DNA structure' as an intermediate of replication. As shown in these studies, the combination of biochemical reconstitution and AFM nano-observation is useful to analyze molecular mechanisms of the DNA-mediated events.

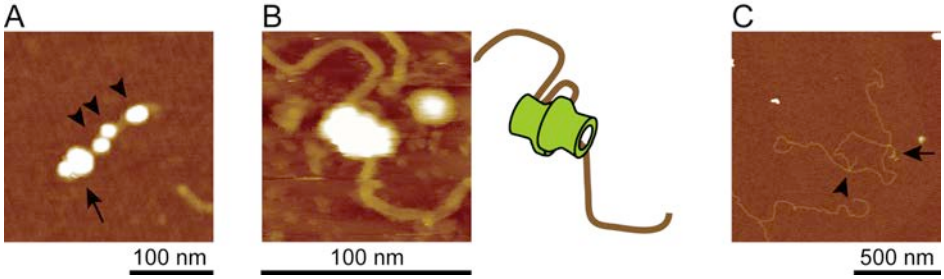

Figure 1. (A) the ORC–chromatin complex was visualized by AFM. The beaded structures of uniform size indicated by arrowheads are nucleosomes, and the larger complex indicated by arrow is the complex consisting of ORC and nucleosomes. (B) The MCM-DNA complex was visualized by frequency modulation AFM (FM-AFM). A schematic representation of the possible architecture is shown in the right panel. (C) The AFM image of the DNA that was partially replicated. The branched DNA structure with ssDNA and chicken foot-like structure are indicated by arrow and arrowhead, respectively.

References:

[1] Concerted interaction between origin recognition complex (ORC), nucleosomes and replication origin DNA ensures stable ORC-origin binding. K. Hizume, M. Yagura, and H. Araki, *Genes Cells.* **18**, 764 (2013).

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[3] DNA polymerase epsilon-dependent modulation of the pausing property of the CMG helicase at the barrier. K. Hizume, S. Endo, S. Muramatsu, T. Kobayashi, and H. Araki, *Genes Dev.* **32**, 1315 (2018).

Natural Sciences at Exeter; Research at the Heart of Undergraduate Teaching

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The Natural Sciences undergraduate degree programmes (BSc, MSci) at Exeter draws from Biology, Physics, Chemistry and Mathematics. The philosophy underlying the introduction of Natural Sciences to Exeter has been to develop a truly cross disciplinary degree programme, teaching our students through exposure to cutting edge research, with a strong emphasis on developing strong practical scientific skills, along with very high academic expectations, nurturing the next generation of researchers, who will need to operate in the very cross disciplinary environment of scientific research today. In Natural Sciences, we place scientific research at the very core of our teaching and project work.

Natural Sciences is a very challenging degree, along with bespoke modules in the first and second year, which cover all disciplines. Most teaching modules taken by our students are those offered by the individual disciplines, e.g. our students in the 2nd year will all take Differential Equations with the Mathematics students or take Quantum Mechanics with the 2nd year Physics students or Advanced Cell Biology with the Biologists etc… Consequently our students have to work at or above the level of the individual disciplines across all the disciplines.

In my presentation, I want to discuss our Natural Sciences degree programmes, particularly focusing on the third year Group Project Module which I lead, as an example of our innovative, research led teaching. This module offers students the opportunity to work with a number of high profile external partners, in addition to research groups from across the university. This module develops a wide range of key skills such as analytical problem solving, teamwork, and organizational skills and scientific communication skills, all of which are vital to becoming a successful researcher. I would like to use my experience of setting up this module to inspire other university teachers/lecturers to think about developing similar approaches to research led teaching to produce the graduates we need to work in cross disciplinary scientific research in the future.

Reference:

[1] http://www.exeter.ac. uk/undergraduate/degrees/natural-sciences/

Nanoparticles for Bioimaging

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Tracking and monitoring of various foreign bodies, including drugs, chemicals, bio/nano materials etc., in a living organism, including humans has always been an extremely challenging task. The actual importance of knowing where a certain entity is in the body, in real-time opens up a huge trove of information that can be used by the biomedical fraternity to further understand the fate and effects of that particular entity. Nanotechnology has had a great impact on the medical scenario since its advent, particularly concerning drug delivery. Yet, the issue of the fate of these drug delivery vehicles remains unresolved. Conventional dyes and contrast media which would emit signals when excited with light, fluorescence, electrons, ultrasound, X-ray, magnetic resonance, positrons etc. were employed for this purpose. The problem with these dyes and contrast media (some radioactive) is that they are usually toxic, have low signal-noise ratio, poor photostability, low quantum yield, insufficient in vitro and in vivo stability, etc. This has triggered a rapid interest in developing imaging moieties that can overcome these limitations.

My talk will focus on a range of highly biocompatible nanomaterials which can be utilized as imaging probes with various excitation sources as mentioned above. Most nanomaterials discussed have multiple imaging potentials.

References:

[1]. Mohamed MS, Veeranarayanan S, et. al. External Stimulus Responsive Inorganic Nanomaterials for Cancer Theranostics, *Advanced Drug Delivery Reviews*, doi.org/10.1016/j.addr.2018.10.007 (2018).
[2]. Veeranarayanan S, Mohamed MS, et. al. Photodynamic Therapy at Ultra-Low NIR Laser Power and X-Ray Imaging using Cu_3BiS_3 Nanocrystals. *Theranostics*, **8**, 5231 (2018).
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