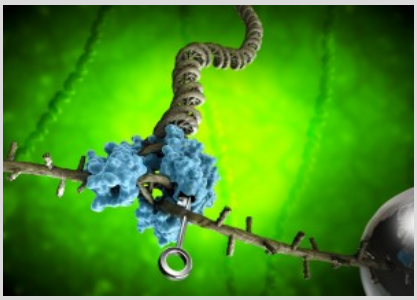
Nynke Dekker Lab

Single-molecule biophysics of DNA and RNA



OPEN STUDENT PROJECTS
2020 - 2021

www.nynkedekkerlab.tudelft.nl

NYNKE DEKKER LAB

The Nynke Dekker Lab (http://nynkedekkerlab.tudelft.nl) is a highly successful research laboratory focused on understanding the key cellular process of **nucleic acid replication** from a biophysical perspective in viral, bacterial, and eukaryotic systems. We perform our studies both using purified components and inside living cells. To study in particular the dynamic aspects of replication, we make use of state-of-the-art **biophysics** (including cutting-edge techniques such as magnetic tweezers, flow-stretched DNA curtains, and super-resolution fluorescence in living cells) that is highly integrated with **biochemistry**. Studying molecular processes using these techniques requires broad expertise; our lab is composed of a multidisciplinary team of international scientists with backgrounds in quantitative biology, (bio)chemistry, or (bio)physics. If you are interested in contributing to a **mechanistic understanding of replication**, there are plenty of opportunities for exciting and challenging BEP/MEP projects available, as described below!

DEPARTMENT OF BIONANOSCIENCES

The Nynke Dekker Lab is located within the Department of Bionanoscience at TU Delft. The Department operates at the interface between cell biology, single-molecule biophysics, and synthetic biology, and as such research in the Department ranges from the functioning of single cells in all their complexity down to the single-molecule level. Understanding the fundamental molecular processes is of crucial importance for diverse developments and applications involved in targeted therapeutics, biomedicine, diagnostics and alternative energy sources, among others. Students in the Nynke Dekker Lab have ample opportunity to participate in the scientific and social events organized at the Departmental level.

BECOMING A STUDENT IN THE NYNKE DEKKER LAB

What types of students are we looking for?

How does it feel create knowledge that will change textbooks for the next generation? If you are curious and strongly motivated to generate new knowledge, and willing to learn how to systematically do so, then you are a qualified applicant. With a background in the disciplines of either molecular biology, genetics, biochemistry, biophysics, physics, or informatics, you will have much to contribute to our team-oriented, multidisciplinary studies. You can also simultaneously develop an expertise in another area while working with us. Most importantly, you will have the opportunity to develop your own ideas and hypotheses and thereby grow into an independent scientist or professional. As such, you will be accorded equal respect and responsibility to any group member.

What do we offer?

Students are fully integrated into our multidisciplinary teams, gaining a unique experience at the frontier of research. The projects include all aspects of practical lab work, experiments, and analysis. Every student will be guided to grow in his/her planning, experimental, and presentation skills and become independent to a level where he/she can make a real contribution to research at the cutting edge of science, often including a publication in an international scientific journal. Typically we have several students in the group, allowing you to interact with your fellow students scientifically as well as socially.

When can you start?

You can start at any time of the year. At present, we would love to recruit MEP students who would like to begin at the start of the summer, or at the start of the fall semester. However, BEP students are also welcome. Generally speaking, the lab attracts many students from the Nanobiology program starting in February. If you too would like to start during this period, please contact us well in advance to secure a spot.

How can you find out more about the projects?

We are driven by the idea that science, fun and intellectual freedom are inseparable. Do you want to know in detail about our research and projects, you are welcome to come for a coffee discussion and also take a lab tour! You may directly contact any of the PhD students/postdocs listed on the specific projects below, or e-mail Dr. Belen Solano Hermosilla (Research Manager, b.p.solanohermosilla@tudelft.nl) or Prof. Nynke Dekker (Group Leader, n.h.dekker@tudelft.nl) for a more general overview.

CURRENT BEP AND MEP PROJECTS

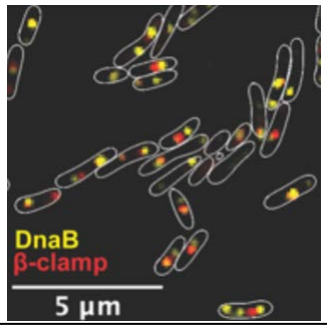
1. Live cell imaging: understanding life and its processes by microscopy

All that life has to offer in function and diversity has a basis in how different cells and their components work together. The genetic information of the cell, DNA, provides a crucial template for all this, and any damage to this coded information can be detrimental to the organism.

- What is the goal of our research?

Our broad interest lies at the heart of understanding how the cell responds to DNA damage. The cell has the ability to harness many DNA repair pathways, depending on the type of damage, and understanding them in detail opens doors to understanding diseases when the pathways malfunction.

- What tools do we use?



Super-resolution image of an E. coli bacterium containing labelled proteins that emit light at three separate wavelengths.

The bacterium *E. coli* is our experimental workhorse, involving genetic engineering and molecular biology. We use live cell, widefield fluorescence, confocal and super-resolution microscopy approaches to address our research. Interpreting such data relies on in-house and open source algorithms. In summary, in an interdisciplinary fashion we apply principles of physics to unravel biological mechanisms.

- What is the significance to society of what we do?

Antibiotic drug targets: With antibiotic resistance becoming a global phenomenon, our work on understanding bacterial replication and repair provides essential knowledge for the future / **DNA repair and cancer**: We enhance fundamental knowledge of DNA replication, errors in which are linked to the development of cancer / **Data analysis**: We develop novel analyses and algorithms to understand biological processes.

- What will your specific project look like?

We are looking for student colleague(s) interested in exploring how 'accessory helicases' function in the cell. While understood superficially so far, these helicases are believed to be critical players in DNA damage, in addition to the main replicative helicase that we know about. Evolution over thousands of years can be trusted on why a single helicase is not enough. You may choose to focus on either experimental microscopy or on quantitative data analysis

Contacts:
Dr. Belen Solano Hermosilla, (B.SolanoHermosilla@tudelft.nl)
Dr. Edo van Veen, data analyst and programmer expert (*e.n.w.vanVeen@tudelft.nl*)

2. DNA replication: In vitro single-molecule studies of eukaryotic replication

Human beings copy a light-year's worth of DNA in their lifetimes. How this is mechanistically achieved remains under very active investigation.

- What is the goal of our research?

The replication of genomic DNA is one of the core processes that takes place during cell cycle progression and proliferation. It is performed by the replisome, a multi-protein complex that incorporates nucleotides into the genome with high fidelity while advancing and opening the double-stranded DNA. In eukaryotic organisms like ourselves, we still know little about how the replisome's proteins organize and interact in a dynamic manner to duplicate not only our genes, but also the compact chromatin environment in which they are embedded.

- What tools do we use?

Due to recent advances in replisome reconstitution, we now have the opportunity to investigate the mechanics of DNA replication in eukaryotes *in vitro*. We employ state-of-the-art protein purification, protein labeling, fluorescence microscopy, magnetic tweezers, and nanofluidics to characterize the real-time dynamics of the replisome.

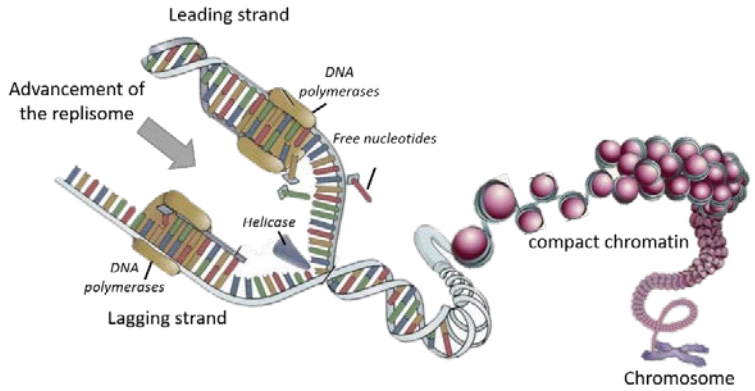
- What is the significance to society of what we do?

DNA repair and cancer: We enhance fundamental knowledge of DNA replication, errors in which are linked to the development of cancer / **Protein labeling:** We develop new proteins that permit the imaging of DNA replication. / **Advanced microscopy and data analysis**: We develop new imaging modalities, analyses, and algorithms to understand biological processes.

- What will your specific project look like?

We are looking for student colleague(s) interested in quantitatively exploring eukaryotic

replication, including in the context of chromatin. While the overall features of eukaryotic replication are known, very little is known about the dynamics. At present, we have several projects available on experimental single-molecule microscopy, and one on quantitative data analysis. On bare DNA, we are studying the kinetics of replisome progression from different perspectives. On chromatin, we are interested in the dynamics of histone displacement in front of the replisome and reassembly behind it, and the role the replisome plays in organizing that process.



Replisome (polymerases, helicase, and other proteins) advancement while replicating DNA in the context of chromatin

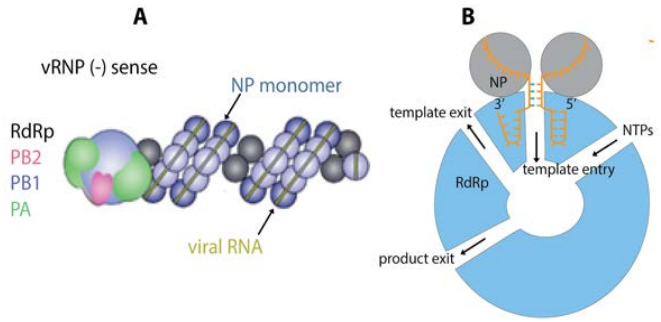
Contact:
Dr. Humberto Sánchez, biochemist/ biophysicist (*h.sanchez@tudelft.nl*)
Dr. Kaley Mc Cluskey, biophysicist (*K.A.McCluskey@tudelft.nl*)
Dr. Theo van Laar, molecular biologist (*t.vanlaar@tudelft.nl*)
M.Sc. Daniel Ramirez, biochemist/ biophysicist (*d.f.ramirezmontero@tudelft.nl*)
Dr. Edo van Veen, data analyst and programmer expert (*e.n.w.vanVeen@tudelft.nl*)

3. In vitro single-molecule studies of RNA-dependent RNA polymerase replication of negative strand RNA viruses.

RNA viruses represent an existing and emerging global threat to human and animal health. Unfortunately, we lack sufficient knowledge of the molecular mechanism of RNA virus replication to specifically target intervention stratgies against these viruses.

- What is the goal of our this project?

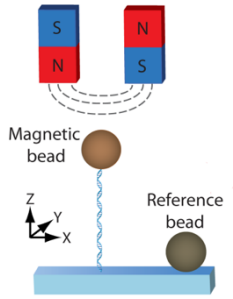
The overarching goal is to create fundamental knowledge on the dynamic molecular mechanisms of negative-strand RNA virus proliferation. Our model system is influenza A virus, a member of the *Orthomyxoviridae* family of viruses. Influenza A viruses outbreaks are on the rise and are driven by the continuing emergence of novel strains. Recent advances in the biochemistry of influenza A virus RNA-dependent RNA polymerase (RdRp) have opened up new avenues for the study of its mechanics and kinetics. With this project, we focus on two key goals: (i) quantitative characterization of the kinetics of influenza virus RdRp during RNA synthesis and (ii) assessment of how local RNA sequence or structure influence these kinetics and potentially lead to nucleotide misincorporation.



The influenza virus RdRp. (a) The assembled vRNP of influenza virus. The RdRp, composed of three subunits PB2, PB1 and PA, interacts with the 3' and 5' ends of the viral RNA, maintaining the vRNA in a circular conformation. The RNA bound to NP forms an helical structure, mainly due to NP oligomerization. (b) Schematic representation of the RdRp bound to the 3' and 5' end of the vRNA, indicating the template entry and exit channels, the NTPs entry channel and the product exit channel.

- What tools do we use?

The large size and complexity of the multi-subunit RdRps often encountered in negative-strand RNA viruses have long hampered their systematic study relative to that of positive-strand RNA viruses. In recent years, however, advances in the biochemistry of these RdRps have permitted the expression and purification of recombinant active forms and subsequent structural determination via crystallization or electron microscopy. Advances have been particularly great for influenza virus RdRps, which were the first of any negative-strand RNA virus to be crystallized. This now makes it possible to, for the first time, gain insight into the molecular mechanisms that underlie the functioning of these enzyme complexes by performing systematic *in vitro* studies. We employ state-of-the-art RdRp purification and labeling, fluorescence microscopy, in-vitro RdRp replication assays using synthetic transcripts in bulk and in single-molecule assays.



The magnetic tweezers as an example of a single-molecule technique. Multiplexed magnetic tweezers are very useful in dissecting polymerase mechanism and will be employed in the study of influenza RdRp.

- What is the significance to society of what we do?

Influenza viruses constitute a constant threat to global health. Despite the impact of influenza viruses, basic understanding of viral replication is still lacking. This proposal constitutes fundamental research aimed at enhancing our understanding of RNA synthesis by the influenza virus RdRp. More generally, the knowledge generated will also pertain to the mechanisms

employed by multi-subunits RdRps of other negative-strand RNA viruses. By providing insights into viral adap tation and evolution mediated through the influenza virus RdRp, this project will also have implications for public health, animal health, and biomedical research.

- What will your specific project look like?

We are looking for student colleague(s) (preferably MEP level) interested in quantitatively exploring influenza virus RdRp replication. You will be optimizing single-molecule assays with recombinant influenza virus polymerase and synthetic constructs to study the replication dynamics. This project will be carried out in close collaboration with Dr. Mathilde Richard, virologist at the ErasmusMC.

Contact:

Prof. Nynke Dekker, biophysicist, N.H.Dekker@tudelt.nl
Dr. Mathilde Richard, virologist at the ErasmusMC, department of Viroscience, m.richard@erasmusmc.nl)