Nanotechnology for single-molecule biology:
from nanopore protein sequencing to chromosome organization

Distinguished Professor Cees Dekker
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WEDNESDAY, 6 NOV, 3PM, LT 32

Moderator : Assoc Prof Slaven Garaj

Abstract

Nanotechnology offers fantastic opportunities to contribute to biology. I will present some recent examples from my lab where nanotech single-molecule tools are used to unravel the biology of cells down to the single-molecule level.

A DNA origami turbine powered by nanoscale flow [1]
We recently built artificial nanoscale turbines. We demonstrated driven rotary motion of a nanoscale DNA origami turbine which harnessed energy from a water flow generated by a static chemical or electrical potential gradient in a solid-state nanopore. The origami nanoturbine consisted of a 6-helix DNA bundle that adopted a chiral conformation upon phoretic docking onto the nanopore and subsequently displayed a sustained unidirectional rotary motion of up to 20 revolutions/s. These artificial nano-engines operate autonomously in physiological conditions, converting energy into useful mechanical work.

Nanopore-based sequential reading of peptides [2]
We recently demonstrated a nanopore-based single-molecule peptide reader capable of reliably detecting single amino-acid substitutions within individual peptides. A peptide is linked to a DNA molecule and sequentially pulled through a biological nanopore by a DNA helicase in single amino-acid steps. Stepping ion-current signals enable discrimination of single-amino-acid substitutions in single reads. Notably, we demonstrated the capability to 'rewind' peptide reads, obtaining indefinitely many independent reads of the same molecule, yielding an undetectably low error rate in single-amino-acid variant identification. Recently, we expanded this concept to discriminating single post-translational modifications within peptides of mixed charge. These proof-of-concept experiments constitute a promising basis for the development of a single-molecule protein sequencer.

Real-time imaging of DNA loop extrusion by condensin and cohesin SMC complexes [3]
Structural Maintenance of Chromosomes (SMC) proteins like cohesin and condensin spatially organize chromosomes by extruding DNA into large loops. Using single-molecule assays, we provided unambiguous evidence for loop extrusion by directly visualizing the processive extension of DNA loops by SMCs in real-time. In recent extensions of this work, we showed how this process occurs on supercoiled DNA, that SMCs also can exhibit phase condensation, and that SMC proteins can bypass huge roadblocks of bound proteins on DNA.

References:

[1] X. Shi et al, Nature Physics 18, 1105 (2022); X. Shi et al, Nature Nanotechnology 19, 338 (2024)
[2] H. Brinkerhoff et al, Science 374, 1509 (2021); I. Nova et al, Nature Biotechnology 42, 710 (2024)
[3] Ganji et al, Science 360, 102 (2018); Kim et al, Nature 579, 438 (2020); B. Pradhan et al, Cell Reports 41, 111491(2022)

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Biography

Cees Dekker is a physicist who moved from quantum solid-state physics to nanobiology. In the 1990s, he pioneered nanotechnology and discovered many of the exciting electronic properties of carbon nanotubes, establishing the first single-molecule transistor. In 2000, he moved to single-molecule biophysics and nanobiology, applying nanotechnology to biological systems and enforcing various breakthroughs from DNA and protein sequencing with nanopores to DNA loop extrusion by novel motor proteins. His current research focuses on nanopores, chromosome structure, and developing synthetic cells. Characteristically, Dekker pioneers new fields and defines new directions, both organizationally in e.g. establishing the Kavli Institute and a new Department of Bionanoscience at Delft and spearheading international initiatives to build synthetic cells, but more importantly also scientifically - by pioneering molecular electronics and nanobiology yielding major discoveries from nanopore sequencing to DNA loop extrusion by motor proteins.

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Lecture series by

Distinguished Professor Cees Dekker

Kavli Institute of Nanoscience, Delft University of Technology, The Netherlands

Lecture 1
WED, 6 NOV, 10AM, LT 32

Nanopores for fingerprinting and sequencing individual proteins

Moderator : Prof Sow Chorng Haur

Abstract

Nanopores are versatile biophysical sensors for single biomolecules. In the past decade, they even have been developed into a technology single-molecule DNA sequencing. Hence, I will first present an overview of nanopore DNA sequencing:

DNA sequencing with nanopores

New efforts are directed towards the fingerprinting and sequencing of proteins at the single-molecule level. In this talk I will present two examples of our recent research in this direction.

Trapping a single protein using a nanopore electro-osmotic trap (NEOtrap)

We developed a new type of single-protein trap, which we name nanopore electro-osmotic trap (NEOtrap). Here, an origami structure is used to create a strong electroosmotic flow in a solid-state nanopore, which allows us to catch a single protein and hold it at the most sensitive region of the nanopore for label-free sensing. This allows to distinguish various proteins, as well as study the dynamics of a single protein for a long time (even hours). Orientation control and linking of the DNA origami sphere can significantly improve the NEOtrap. Recent experiments on dCas9/RNA/DNA allow for modelling the trapping kinetics.

Nanopore-based sequential reading of individual peptides

We demonstrated [2] a nanopore-based single-molecule peptide reader that is capable of reliably detecting single amino-acid substitutions within individual peptides. A peptide is chemically linked to a DNA molecule and sequentially pulled through a biological nanopore by a DNA helicase in single amino-acid steps. Stepping ion-current signals enable discrimination of single-amino-acid substitutions in single reads. Notably, we demonstrated the capability to 'rewind' peptide reads, obtaining indefinitely many independent reads of the same molecule, yielding an undetectably low error rate in single-amino-acid variant identification.

Recently [3], we expanded this concept to discriminating post-translational modifications (PTMs). PTMs play a key role in regulating protein activity and they are the crucial elements underlying the enormous diversity in the proteome. Current mass-spectrometry detection methods cannot measure PTMs in single molecules or differentiate between closely spaced PTM sites. We demonstrated the ability to detect PTMs at the single-molecule level on immunopeptide sequences with cancer-associated phosphate variants by controllably drawing the peptide through the sensing region of a nanopore. We discriminate peptide sequences with one or two closely spaced phosphate PTMs with 95% accuracy for individual reads of single molecules. The data also prompted us to start modelling the traversal of peptides of mixed charge through nanopores. In my presentation I will present the state of the art of these experiments in our lab, which is expanding efforts to detecting a range of other PTMs and developing a workflow to read natural proteins.

These proof-of-concept experiments constitute a promising basis for the development of a single-molecule protein sequencer.

References:

[1] S. Schmid et al, Nature Nanotechn. 16, 1244 (2021); C. Wen et al, Nano Lett. 23, 3, 788 (2023)

[2] H. Brinkerhoff, A.S.W. Kang, J. Liu, A. Aksimentiev, C. Dekker, Science 374, 1509 (2021)

[3] I.C. Nova, J. Ritmejeris, H. Brinkerhoff, T.J.R. Koenig, J.H. Gundlach, C. Dekker, Nature Biotechnology, DOI 10.1038/s41587-023-01839-z (June 2023)

Lecture 2
THURS, 7 NOV, 10AM, LT 32

DNA loop extrusion by the SMC molecular motors that organize our chromosomes

Moderator : Assoc Prof Wang Zhisong

Abstract

Structural Maintenance of Chromosomes (SMC) proteins like cohesin and condensin spatially organize chromosomes by extruding DNA into large loops. We discovered many of the intriguing properties of this new class of DNA-translocating molecular motors by directly visualizing the processive extension of DNA loops by SMCs in real-time [1]. Single-molecule methodologies have since then provided many new insights into the structure and function of this novel class of DNA-processing molecular motors [2]. Strikingly, SMCs can easily bypass DNA-binding proteins on DNA, even ones that exceed the size of the large ring-shaped SMCs – demonstrating that loop extrusion occurs nontopologically. Due to biochemical interactions, however, the DNA-binding protein CTCF can stop SMC traversal, which makes it define topologically associating domains in chromosomes.

In my presentation, I will present the most exciting latest experiments from our lab. Magnetic tweezers resolved the force-dependent step size and identified ATP binding as the step-generating process in DNA loop extrusion by condensin [3]. Surprisingly, we recently found that all SMCs induce 0.6 negative supercoil into the extruded loop in each individual step of the loop extrusion [4]. These findings have direct implications for modelling these intriguing floppy molecular motors. Using a single-molecule visualization assay, we extensively studied how SMC proteins interact with DNA-binding proteins that potentially act as roadblocks to loop extrusion. Strikingly, we found that SMCs can easily bypass most proteins roadblocks on DNA [5], even ones that exceed the size of the large ring-shaped SMCs – demonstrating that loop extrusion occurs nontopologically (i.e., without DNA passing through the lumen of the SMC). Due to biochemical interactions, however, the DNA-binding protein CTCF (which is the key element defining topologically associating domains in chromosomes) was found to asymmetrically block loop-extruding cohesin, in a process which was found to be dependent on DNA tension. The data show that CTCF is not, as previously assumed, simply a barrier to cohesin-mediated loop extrusion but an active regulator of this process. Finally, we have discovered that the cohesin subunit NIPBL is a direction switch for the cohesin which is a 1-side DNA loop extruder [7].

Overall the results reveal mechanistic principles of how SMCs regulate the genome architecture.

References:

[1] Ganji et al, Science 360, 102 (2018)

[2] Kim et al, Nature 579, 438 (2020); C. Dekker et al, Science (under review)

[3] Ryu et al, Nucl. Acid Res. 50, 820 2022)

[4] Janissen et al, Nucl. Acid Res., under review

[5] B. Pradhan et al, Cell Reports 41, 111491(2022)

[6] Davidson et al, Nature 616, 822 (2023)

[7] Barth et al, under review (2024)

Lecture 3
FRI, 8 NOV, 10AM, LT 32

Building a synthetic cell from the bottom up

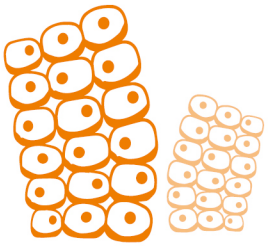
Moderator : Prof Sow Chorng Haur

Abstract

In a futuristic project, we have started to explore the boundary between nonlife and life, by attempting to build a living cell from lifeless components. I will start my talk by providing some historic context and describing new national programs in the Netherlands that aim at building such a synthetic cell.

We are currently devising first steps aimed at ultimately realizing live synthetic cells that are constituted from lifeless biomolecules in a bottom up fashion. In this talk I will present some elements of that, viz., the definition of liposome containers, the building of a minimal divisome, and the spatial organization of a synthetic genome.

I will recap our various microfluidics strategies to encapsulate proteins in liposomes. I will describe our efforts where we explored various proteins that might constitute a minimal divisome, i.e., a minimal set of proteins that can establish the division of a mother liposome into daughter liposomes. I will present recent results on the bacterial protein Dynamin A (DynA) where we used FRAP fluorescence microscopy to study in vitro reconstituted DynA inside liposomes. Upon external reshaping of the liposomes into dumbbells, DynA self-assembled at the membrane neck, resulting in membrane hemi-scission and even full scission. DynA proteins were found to constitute a simple one-component division machinery that is capable of splitting dumbbell-shaped liposomes, marking an important step towards building a synthetic divisome.



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