Several master thesis projects are available at the NanoDynamicsLab

Check the descriptions below and
get in touch by email schmid@nanodynlab.org
if you're interested in joining our team.

We look for highly skilled, motivated, and friendly students
with diverse backgrounds (life sciences / chemistry / physics), and
with an intrinsic drive for innovative single-molecule biophysics
to study life at the molecular level.

Be courageous: we enjoy teaching you new stuff, and
we try to tune projects according to your interests.
But don't come if your main interest is on macroscopic objects or
on kit-based biochemistry.

Please also note: all projects are part of ongoing research, hence the available projects will change over time.

-> Get in touch for more specific details:
please mention which projects you're most interested in and WHY.

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Project 1: **Nano-cavities for new & improved single-molecule FRET**

Single-molecule FRET is a uniquely powerful technique to resolve protein interactions and protein conformational changes, even at the single-molecule level! A current limitation is, however, the very low nano-molar concentration that is usually needed to resolve *single* molecules. This limits the study of many highly relevant low-affinity interactions that e.g. control the function of chaperone proteins. To solve this problem, we are developing a new detection scheme (Figure 1): we use 100nm small nano-cavities – so-called zero-mode waveguides – to overcome the typical concentration limit in single-molecule FRET studies, with the goal to observe in real-time the formation and dissociation of protein complexes in the Hsp90 chaperone system.

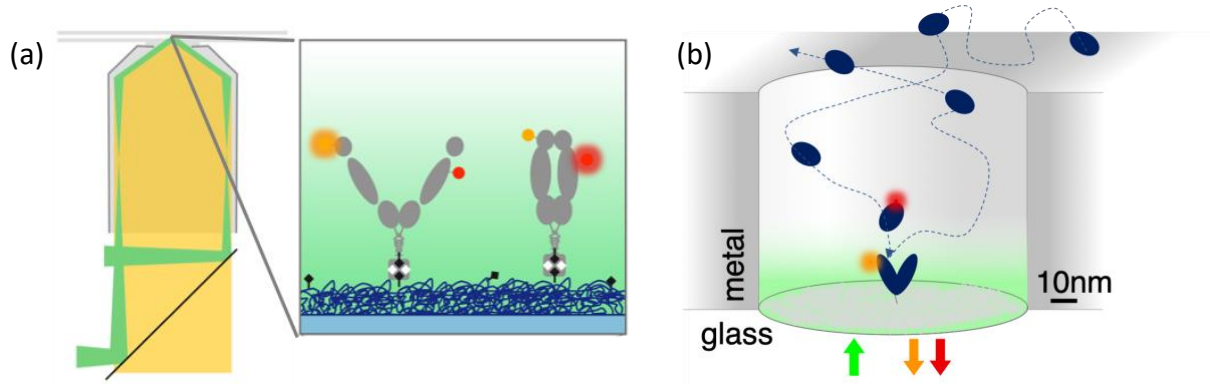

Figure 1: (a) Conformational changes in single proteins are revealed by FRET and TIRF microscopy, here illustrated with a cross-section view through the microscope objective and a zoom view of the coverslip at molecular scale. (b) Low-affinity interactions will be studied using advanced zero-mode waveguides where only molecules in tiny wells are visible, while those in the background stay dark. This enables single-molecule resolution at physiological

The goal of this project is to use the newest generation of zero-mode waveguides (zmw) to overcome the traditional concentration limit in single-molecule FRET, and thereby enable the study of many essential low-affinity complexes. You will get to play with these sophisticated zmw nanostructures (Figure 2), and watch single molecules come and go in real-time.

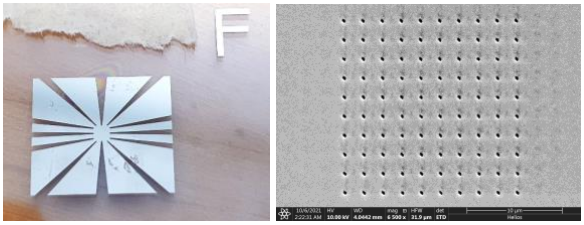

Figure 2: Zero-mode waveguides photo (left) and electron-micrograph (right) with 200nm diameter cavities. (Images courtesy of Caneva Lab, TU Delft.)

- You will observe how biomolecules (DNA or proteins) interact at the single-molecule level.
- You will use surface chemistries to create functionalized, bio-compatible zero-mode waveguides.
- You will learn to independently run & control single-molecule FRET experiments.

Project in collaboration with Sabina Caneva, 3mE, TU Delft.

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Project II: **Direct observation of the photocurrent of PhotoSystem I**

Photosynthesis powers virtually all life on earth, and Photosystem I (PSI) is the most efficient molecular machine on the planet. Typically, PSI function is measured using fast optical spectroscopies. However, the central function of PSI is to convert light energy into a transmembrane potential. Therefore, we aim to establish a much more direct *electrical* detection of PSI's function, bearing the potential for time-resolved single-molecule recordings of PSI activity.

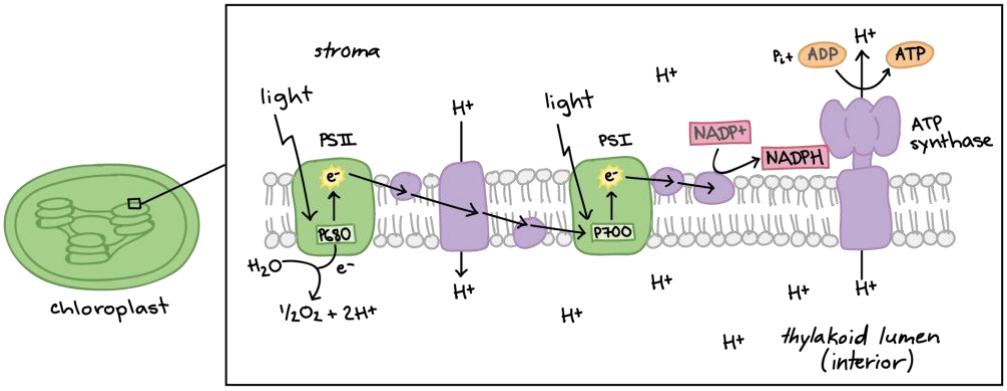

Figure 1: PSI functions in the linear electron transport chain together with PSII. PSII uses light energy to split water into electrons, protons and oxygen. PSI accepts the electrons and uses light energy to generate the reducing power to convert $NADP^+ + H^+$ into NADPH.

The goal of this project is to directly measure the photocurrent (more specifically, the electrical membrane potential) produced by PSI under light exposure. To this end, you will purify PSI from spinach, embed it into a free-standing lipid bilayer separating two buffer reservoirs, and detect the potential change upon light exposure. Establishing this most direct photo-current detection represents a big step forward that may reveal new functional features, such as bursts and pausing of single PSI molecules that are undetectable using existing bulk techniques.

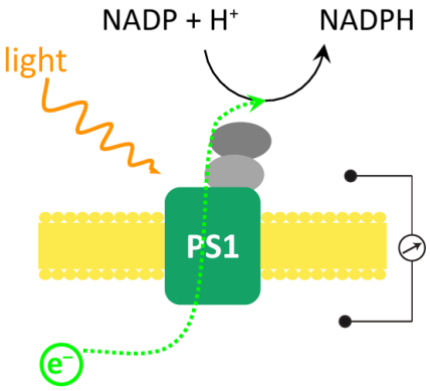

Fig. 2: A minimal system for the direct observation of the photocurrent of PSI embedded in a free-standing lipid bilayer.

- You will learn to purify PSI from spinach.
- You will learn to build free-standing lipid bilayers as used in nanopore sequencing technology and run corresponding electrical recordings.
- You will develop a new and most direct detection scheme for photosynthetic activity with the potential to enable radically new experiments leading to previously inaccessible discoveries.

Project in collaboration with Emilie Wientjes (BIP)

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Project III: **Super-resolution imaging of bacterial microcompartments**

Did you know that bacteria have organelles? Yes, they do and some of them are called Bacterial Micro-Compartments (BMCs). BMCs are proteinaceous organelles that facilitate efficient metabolism in bacteria and protect them against toxic reaction intermediates (Figure 1). Many questions remain open on how BMCs can do what they do, in particular regarding import and export of compounds into/out of BMCs.

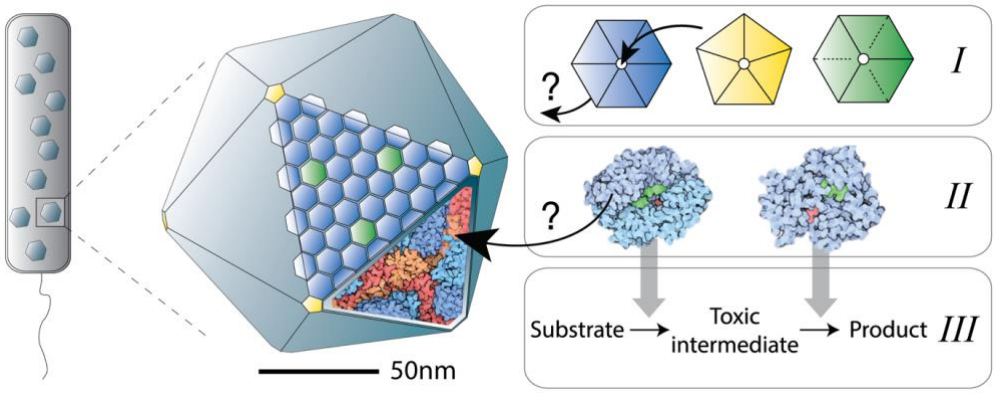

Figure 1: Bacterial microcompartments are 100-400nm protein shells that are filled with specific, encapsulated enzymes. Pressing research interests include: (I) import and export through nanopores in their protein shell (similar to viral capsids); (II) control of enzyme encapsulation; and (III) re-purposing BMCs for green biotech applications.

The goal of this project is to gain a better molecular-level understanding of specific enzyme encapsulation into BMCs. To this end, we fuse an encapsulation peptide (EP) to a fluorescent reporter protein (FP). You will use advanced fluorescence microscopy to image BMC-encapsulated FPs in Clostridia, to obtain a more precise image of the encapsulation efficiency, the number of encapsulated molecules per BMC, and the heterogeneity or homogeneity between individual BMCs.

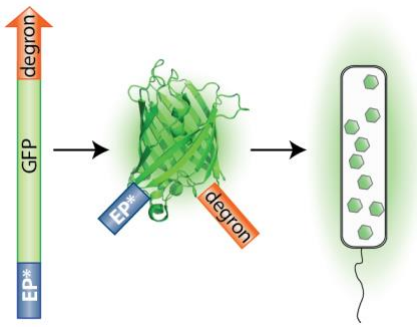

Figure 2: GFP-fused to an encapsulation peptide (EP) to monitor BMC encapsulation in Clostridium beijerinckii.

Altogether, we offer you to investigate one of the most pressing questions in current research on BMCs and their application to produce industry-relevant chemicals. This project is an interdisciplinary collaboration between the laboratories of microbiology (Kengen group) and biophysics (Hohlbein & Schmid groups) at WUR.

Interdisciplinary skills that you will obtain in this thesis:

- Hands on experience with *Clostridium beijerinckii,* an anaerobe non-model organism, next to the regular aerobe model organism *E. coli*.
- Plasmid construction for the expression of fluorescent proteins (FPs).
- Advanced *in vivo* imaging technologies, such as super-resolution imaging.
- Quantitative image analysis to elucidate the molecular basis of BMC encapsulation.

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Project IV: **Chaperone chimeras à la Frankenstein studied by smFRET**

To perform their essential functions *in vivo*, proteins rely critically on proper folding. So-called chaperone proteins have evolved to assist in protein folding and activation. Until today, the molecular basis of this assistive action has remained unclear in many cases. Here we employ single-molecule FRET to shed light on the chaperone activity of the heat-shock protein 90 (Hsp90), a central player in protein homeostasis. Interestingly, bacterial and eukaryotic Hsp90 differ drastically, e.g. in the nucleotide dependence of their conformational dynamics. We are interested to identify what causes this striking difference in nucleotide dependence between two structurally very similar proteins. We will create eukaryote/prokaryote chimeras and study the chimera dynamics using single-molecule FRET to identify the minimal functional elements capable of chemical to mechanical energy conversion.

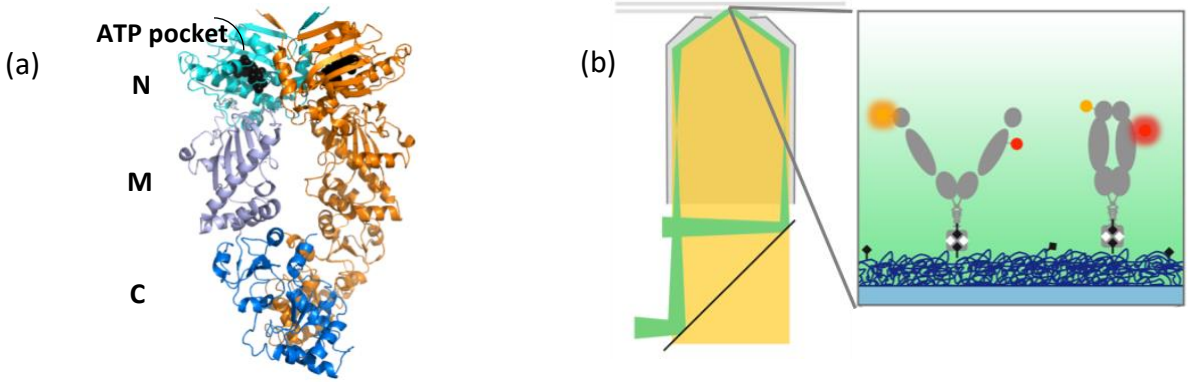

Figure 1: (a) The heat-shock protein Hsp90 is a homo-dimeric chaperone with an exceptionally slow ATPase function. The closed conformation (crystal structure 2cg9) is shown with nucleotide binding domain (N), middle (M), and C-terminal domain (C). (b) Conformational changes in single Hsp90 proteins are revealed by FRET and TIRF microscopy, here illustrated with a cross-section view through the microscope objective and a zoom view of the coverslip at molecular scale.

The goal of this project is to identify the crucial elements that dictate Hsp90's function. Specifically, we ask: which bacterial elements do we need to insert into eukaryotic Hsp90, to re-establish bacterial behavior, meaning the coupling of conformational change to ATP hydrolysis. We anticipate to reveal so far unknown mechanisms with sub-molecular precision.

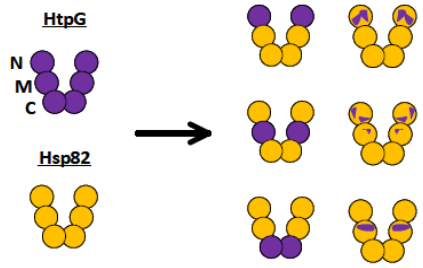

Figure 2: Protein chimeras between the bacterial homologue (purple) and the eukaryotic homologue (yellow) will be created to inform on the key elements that couple energy consumption to conformational

- You will learn to express & purify Hsp90, including biochemical quality control.
- You will learn to independently run & control single-molecule FRET experiments.
- You will learn to use an enzyme-coupled ATPase assay to assess Hsp90's hydrolysis rate.

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Project V: **Exploring the NEOtrap with CRISPR/Cas9**

We recently developed a new single-molecule technique, the Nanopore Electro-Osmotic trap (NEOtrap)[1], to trap and sense single proteins in solution using nanopore and DNA-origami technologies (Figure 1). As a label-free single-molecule technique, the NEOtrap bears great potential for the identification and conformation-sensitive monitoring of unmodified proteins in solution with applications in single-molecule proteomics, enzymology, biosensing etc.[2]

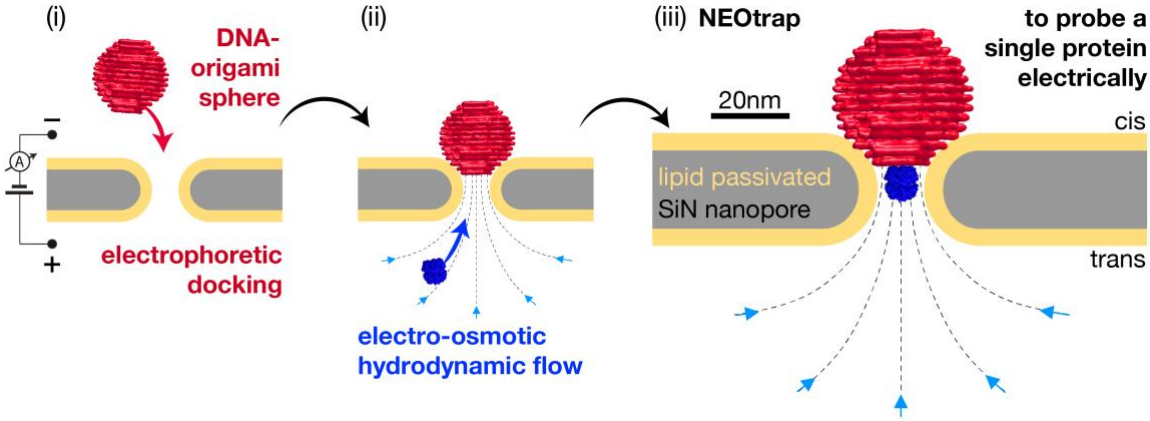

Figure 1: The NEOtrap is formed by docking a DNA-origami sphere onto a solid-state nanopore, which itself creates a hydrodynamic trap for single proteins that are monitored electrically without the need for artificial labels.

The goal of this project is to explore and quantify the trapping potential of the NEOtrap with respect to adverse forces leading to protein escape. Understanding these fundamental processes is key to the interpretation of protein trapping signals, and thus required for protein identification and single-molecule proteomics applications. Cas9 will serve us as a model protein, whose charge can be arbitrarily tuned in corresponding guide RNA and DNA complexes (Figure 2).

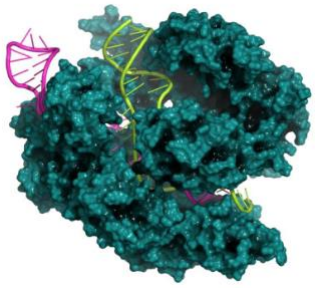

Fig. 2: The CRISPR/Cas9 complex allows one to tune the net charge of the ribonucleoprotein at will.

- You get to learn one of the newest single-molecule techniques (Nature Nanotechnology 2021).
- You will design and build a CRISPR/Cas9 system with varied global net charge.
- You will be the first to trap and sense single Cas9's label-free using the NEOtrap.
- You will quantify the electro-osmotic trapping potential compared to electrophoretic forces.
- You will potentially explore conformational changes in Cas9.

[1] Nanopore electro-osmotic trap for the label-free study of single proteins and their conformations. (2021) *Nature Nanotechnology*

[2] The NEOtrap – en route with a new single-molecule technique. (2021) *iScience*

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Project VI: **Monitoring the auxin hormone pathway using Nanopores**

The nuclear auxin signaling pathway controls plant growth and is fundamental for all land plants. Quantitative information on auxin-controlled protein-protein- as well as protein-DNA interactions are crucial for a complete understanding of how this fascinating plant hormone works (Figure 1). In this project, we focus on the binding of the auxin-dependent transcription factor to its DNA binding site, and we use revolutionary nanopore technology to reveal such protein-DNA binding events *at the single-molecule level*. As explained below (Figure 2), we will literally count bound vs. unbound molecules one-by-one, to obtain quantitative dissociation constants for a library of different DNA sequences.

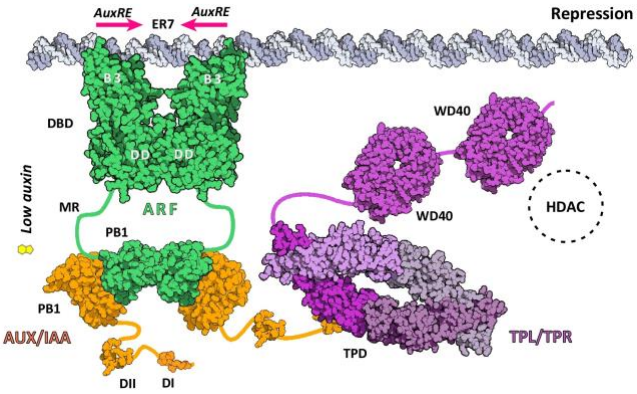

Figure 1: auxin response factor (ARF) is central to plant growth control via the auxin signaling pathway. ARF binds to DNA to control transcription in an auxin-dependent way. [Model by Dinesh (2015) Trends Plant Sci.]

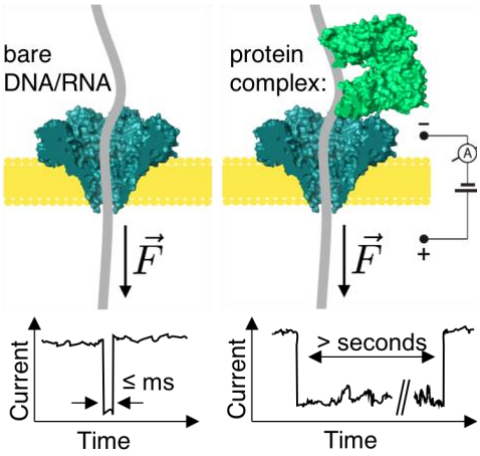

Figure 2: Electrical nanopore detection of DNA molecules (left) and nucleo-protein complexes (right) The latter cannot fit through the pore, leading to much longer pore blockage as observed in the current.

The goal of this project is to monitor ARF transcription factor binding using protein nanopore recordings. DNA translocations through the nanopore will be observed as short dips in the nanopore current (Fig. 2 left), while ARF-DNA binding prevents the free passage of DNA: the nucleo-protein complex gets stuck in the pore which is observed as a much longer blockage of the nanopore (Fig. 2 right). This allows us to distinguish both cases at the single-molecule level and in a label-free way.

- You will get to know protein nanopore technology (related to nanopore sequencing).
- You will get to know the crucial molecular steps in auxin-dependent plant growth control.
- You will be part of a young team that tackles biochemical questions using cutting-edge single-molecule tools.

Project in collaboration with Jan-Willem Borst and Carlo van Mierlo (BIC).

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