Robustness by design? Structural analysis of dynamic metabolic models

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Expertise for the Project: Dynamical systems, systems and control theory, systems biology	Expertise for the Project: Bioreactor cultivation, metabolomics, deterministic modeling

SUMMARY:

Dynamic metabolic models are describing the cellular responses of microorganisms under changing environmental conditions. Despite the enormous extracellular perturbations, robustness is observed, as important quantities, like the cell energy charge, do not react to the dynamics and remain practically constant. We propose a combined experimental and theoretic approach to rigorously investigate the structural sources of this robustness, as well as the trade-off between metabolic robustness and efficiency, and experimentally validate our mathematical findings. Identifying structures and mechanisms that guarantee robustness by design will streamline the synthesis of robust and efficient cell factories (GMO's) for the production of metabolites.

Introduction & scientific questions

Microbial life is constantly exposed to **dynamic environmental conditions** – temperature, nutrient supply and pH **vary in time**. Microbial cells appear to be **robust towards many perturbations**, like substrate pulses or changes in oxygen supply. How this robustness is generated from kinetic and stoichiometric properties, and which trade-offs between **efficiency and robustness** occur, remains to be elucidated. Mathematical tools are being developed to assess whether a dynamical system exhibits a **peculiar behaviour regardless of specific parameter values,** i.e.: robustness is guaranteed **by design**, in view of the **system inherent structure**.

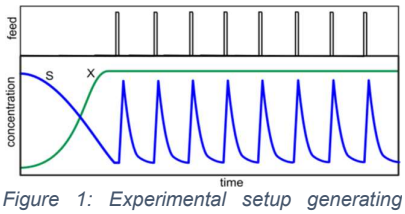

Figure 1: Experimental setup generating rapid feast/famine conditions. The blockwise feeding (20s) leads to gradients in substrate concentration (S). X is the biomass

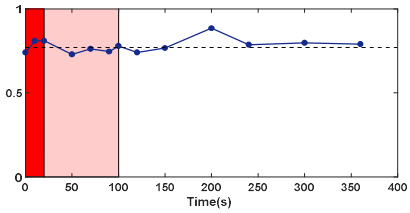

Figure 2: Energy charge (EC) during a feast/famine cycle, which is very stable although the extracellular environment (Fig. 1) changes more than 50-fold. The red region is the feeding phase (20s) and the pink is the feast phase.

Dynamic experiments with repetitive perturbations show that **biomass and product yields are reduced** compared to less dynamic conditions. Yet, even in a bioreactor subject to periodic feeding, resulting in feast/famine cycles (Figure 1), some fundamental values, such as the **energy charge** of the cells, remain surprisingly **constant** even though drastic changes occur in their environment (Figure 2). In our joint project the aim is to compare different organisms with different metabolic kinetics, using a model-based approach, that will help us address the following questions:

- Can **models** reproduce and quantify the **robustness towards perturbations** that is suggested by experimental observations?
- Which **parameters and structures** are essential for a strong robustness?
- Which mechanisms ensure a **constant energy charge** under highly varying flux conditions?

Approach – Duo student work-package

(1) Develop **reduced mathematical models** from published kinetic models (LST)
(2) **Analyse** the underlying interaction structures and **assess robustness** to parameter variations with mathematical methods tailored for the **structural analysis of dynamical systems** (DCSC)
(3) Identify putative mechanisms that can lead to **stable energy charge ratios** (current models are unable to reproduce this feature)
(4) Identify environmental conditions (e.g. cycle length/perturbation intensity) that increase **yield loss**
(5) **Validate** model predictions **experimentally**
(6) Identify mechanisms and **structures** that could increase/ensure **robustness**
(7) Exploiting the insight achieved through the structural analysis, design a **robust-by-design Genetically Modified Organism** to form a product (example: 1,4-butanediol)

Our innovative approach will provide new insight into dynamic phenomena in large-scale bioreactors by bringing together the strong experimental expertise of the Wahl group and the novel mathematical tools for structural analysis recently developed by the Giordano group.

Collaboration benefits

The Giordano group and the Wahl group have complementary skills that are essential to perform this project – unique theoretical approaches for the analysis of dynamic systems and experimental expertise for calibration and validation of models. Modelling techniques and experimental methods for model validation (LST) will be profitably combined with mathematical methods for the robustness analysis of dynamical systems, time-scale separation and structural properties (DCSC).

The project is ambitious from both a theoretical and an experimental point of view, therefore we propose to rely on the collaboration between two students from Life Science and Technology and Systems and Control. This generates a unique opportunity to collaborate with complementary skills, broaden their education portfolio and pursue an exciting multidisciplinary goal.

The BioDate funding will enable us to disseminate the results of this joint project at conferences and perform the respective validation experiments. We believe that this project will have a significant impact on the systems biology community, generating new knowledge on the dynamics and the structural features of cellular networks, and new approaches for data integration. It will also give us a chance to start a fruitful long-term collaboration between the two groups, focused on projects that tackle challenging biological problems with a combined theoretical and experimental approach.

BioDate 2018 MSc project proposal

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TITLE: Squeezing the tube: does enzyme localization accelerate membrane synthesis during division?

SUMMARY: In bacteria, it is commonly assumed that metabolism continues unabated during cell division, i.e., synthesis of proteins and membranes proceeds at the same rate as during cell elongation. However, several decades-old reports suggest that during bacterial division, the rate of membrane synthesis increases two-fold (1). These reports received scant attention until recently, after the discovery of cell cycle-dependent oscillations in electron carriers (e.g. NADPH) that compare closely with membrane synthesis rate oscillations (2). But what could drive such oscillations?

The first enzyme in the *E. coli* phospholipid synthesis pathway, PlsB, determines the overall rate of membrane genesis. Recently, the GB lab has quantified pathway enzymes, substrates, and phospholipid flux, and have concluded that they are highly unlikely to fluctuate with cell cycle progression. Thus, a non-metabolic cue likely accelerates membrane synthesis during division.

The two anionic phospholipids found in the *E. coli* membrane, phosphatidyl-glycerol (PG) and cardiolipin (CL), dramatically activate PlsB *in vitro* (3). CL and PG are enriched at curved membrane surfaces, such as the cell poles (4). If PlsB localizes to the nascent cell poles during division, this could provide a simple explanation for the sudden increase in PlsB activity upon cell division: membrane invagination creates localized curvature enriched with CL and PG, triggering PlsB localization to those CL and PG-enriched regions, increasing its activity. Phospholipid enzymes localize to the divisome of other bacteria (5)*;* however this has not yet been observed in rod-shaped bacteria like *E. coli,* nor is localization connected with cell division-triggered increases in phospholipid synthesis in any bacteria.

We **hypothesize** that localization of phospholipid synthesis enzymes to the nascent cell poles triggers an increase in phospholipid synthesis during cell division.

Proposed work. *Months 1-2:* in the GB lab, the student will use genetic manipulation to prepare *E. coli* strains in which enzymes of phospholipid synthesis have been modified with the HaloTag, a small peptide which binds covalently to chloroalkane-modified fluorescent dyes (6). The student will confirm that the tags do not perturb phospholipid metabolism. *Months 3-7:* in the CS lab, the student will perform live-cell microscopy as well as superresolution imaging to localize tagged phospholipid synthesis enzymes throughout the cell cycle. Observation of division-induced localization of phospholipid synthesis enzymes will provide powerful support to our hypothesis, and preliminary data for a grant application to continue the work in both labs.

Complementarity of project. This project brings together complementary expertise of both groups perfectly: localization effects are invisible on the cell culture level as observation requires single-cell resolution, whereas metabolism is extremely difficult to study using microscopy.

Feasibility of project. The GB lab regularly performs genetic modifications of *E. coli* strains, and superresolution of individual proteins using the HaloTag in live cells is routinely accomplished in the CS group. Issues may arise due to tagging of proteins affecting function; this will be quickly detected upon analysis of phospholipid metabolism and different tagging approaches will be attempted.

Added value to education. The student will emerge with hands-on experience in metabolomics and super-resolution techniques: two fields which are rarely merged and are in extremely high demand.

Impact. A positive result, i.e. demonstrating that enzymes of the phospholipid synthesis pathway localize to the cell poles during division, would strongly suggest that enzyme localization drives an increase in phospholipid flux. This work could lead to the first mechanism of cell cycle-driven metabolic oscillations in bacteria.

A negative result would help to rule out localization as the underlying mechanism of accelerated membrane synthesis during division, and would guide the search for alternative signals.

References

1. Carty CE, Ingram LO (1981) Lipid synthesis during the Escherichia coli cell cycle. *J Bacteriol* 145(1):472–478.
2. Zhang Z, Milias-Argeitis A, Heinemann M (2018) Dynamic single-cell NAD(P)H measurement reveals oscillatory metabolism throughout the E. coli cell division cycle. *Sci Rep* 8(1). doi:10.1038/s41598-018-20550-7.
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BioDate 2018 MSc Project Proposal

Optimal control of combined chemo-radiation therapy treatments for improving cancer care

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Expertise for the Project: Systems and control theory, dynamical systems, optimal control, optimisation

Introduction. In the treatment of cancer patients, chemotherapy and radiotherapy both play fundamental roles, either as standalone modalities, or - in many cases - in combination. For *planning radiotherapy treatments*, the development of sophisticated computational models and computer algorithms has long been a focus to *maximize the therapeutic effect* of using radiation to kill tumor cells, while sparing healthy tissues as possible. Comparatively, the *modelling and optimization of chemotherapy treatments* is largely unexplored, while the clinical application of algorithmic planning methods for individualized chemotherapy is practically non-existent. Similarly, *combined chemo-radiotherapy treatments* are routinely prescribed as a separately optimized radiotherapy treatment with the addition of generic chemotherapy regimen. At the same time, cancer treatments advance in numerous aspects, such as the increasing use of significantly shorter treatments (e.g. 5 treatment fractions instead of traditional 30/35 fraction treatments), or our increasing understanding of the importance of drug and/or radiation resistant cell sub-populations in tumor recurrence.

Scientific challenge. The main goal of this project is to explore how *methods from systems and control theory can aid the planning and optimization of single modality radiotherapy, as well as of combined chemo-radiotherapy treatments*. While some preliminary work exists in pure chemotherapy application, regarding the optimal dosage of a set of chemotherapy drugs to control heterogeneous tumors (consisting of multiple species of cancer cells having varying drug resistance, mutation rate and spatial migration rate), application to the *temporal optimization of radiotherapy or chemo-radiotherapy* is an entirely new idea. With the growing emphasis on shortening treatments and measuring tumor and normal tissue response (by quantitative imaging during treatment), this approach could be vital for *effective treatment adaptation* by choosing optimal times to switch between chemotherapy drugs, or to use radiotherapy in combination or as boosts to certain chemotherapy resistant areas, in order to *achieve tumor control with minimal side effects*.

Specific research plan. As this project aims to *lay down the basics of a completely new research line*, the primary goal is the investigation of how *treatment efficacy can be optimised using control-theoretic methods together with temporal radiotherapy models*, such as the biologically effective dose model and different tumor growth and dose response models, *as well as combined chemo-radiotherapy models*. Different problem formulations will be studied as well as several approaches and algorithms to find their optimal solutions, including Pontryagin's Principle, Hamilton-Jacobi-Bellman equations, and convex and non-convex optimization. For each type of model representing possible combination therapies, the expected outcome is a *systematic approach to suggest clinically viable treatments able to minimize the tumor mass over a finite (time) horizon and minimize the side effects*, by prescribing the optimal starting time and optimal duration of each phase of the therapy.

Student profile. The project offers excellent opportunities for an enthusiastic *math or applied physics student*, keen on both theoretical and numerical work, to perform *interdisciplinary research* and to *learn about current radiotherapy and chemotherapy practices, modelling approaches in medical physics* as well as *optimization methods and approaches from systems and control theory*.

Time planning for the MSc project. *M1-2:* Literature study on radiotherapy and chemotherapy modelling, and on optimal control theory. *M3-5:* Radiotherapy temporal optimization (varying tumor response, growth). *M6-8:* Combined chemo-radiotherapy optimization (additive effects, interplay between chemo and radiotherapy, e.g. radiosensitization). *M9:* Write thesis.

Collaboration benefits and impact. *Integrating state-of-art therapy models* and *control approaches tailored to design optimal treatments is a first step* allowing us to study and demonstrate the benefits of this novel, systematic approach. The *BioDate funding would enable discussing and disseminating the results* of this joint project with the wider clinical research community (e.g. at conferences), *thus initiating a long-lasting collaboration that* combines the complementary expertise of the two groups with practical insight of clinical experts, and *can ultimately improve the life of cancer patients*.

BioDate 2018 MSc project proposal

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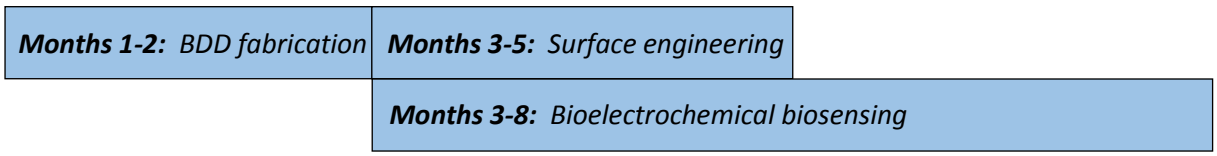
TITLE: Brilliant biosensors

BACKGROUND: Electrochemical biosensing has become a powerful tool to probe and quantify biomolecules. More recently, the focus has turned to toxins and evaluation of the presence of pathogens in fluids, and is becoming of critical importance in clinical analysis and diagnostics, food safety control, environmental monitoring, and drug screening. A crucial component of an electrochemical biosensor is the electrode material, as it defines the measuring sensitivity, the complexity and cost of fabrication, the selectivity to the analyte, surface area, and the stability/durability of the electrode itself. B-doped diamond (BDD) outperforms conventional electrodes in terms of chemical stability, wide solvent window, low noise, resistance to fouling, biocompatibility, surface tuneability for nanostructures, selective surface chemistry, and self-cleaning features. Here, we propose to combine the superior properties of BDD electrodes with the merits of biosensors, such as specificity, sensitivity, and fast response towards a wide range of target biomolecules. This approach will lead to high sensitivity electrochemical sensors for bioelectrochemical detection of a wide range of biological substances (e.g., alcohol, glucose, bacterial infection markers). This would pave way to a novel and sustainable line of research on diamond-aided biosensing at TU Delft, a unique activity that integrates the expertise on micro-fabrication and surface/materials engineering at 3mE with the bioelectrochemistry and enzymology expertise at TNW.

PROPOSED PROJECT: A joint MSc student will develop a first prototype of a biosensor chip comprising of diamond electrodes. He/she will manufacture thin-film BDD electrodes with improved performance and selectivity as compared to conventional bulk diamond electrodes through the nanostructuring (i.e. increasing surface area) by top-down or bottom-up fabrication methods (thin-film diamond growth, micro/nano-patterning). Functionalization with bioorganic molecules such as thiols, allows the selective binding of organic molecules, such as proteins, with orientational control. This will be achieved using self-assembled monolayers with either hydrophobic/hydrophilic or positive/negative solvent exposed head groups. He/she will investigate the structure, composition and performance of thus produced biosensor prototype by advanced materials and electrochemical characterization techniques (SEM, AFM, Raman, electrochemical impedance spectroscopy) present at 3mE and TNW. The student will then apply this to known enzmatically mediated and small molecule bioelectrochemical reactions such as alcohol dehydrogenase turnover and quinone redox. Comparison between flat gold, and BDD electrodes will be used to investigate effective surface area (sensitivity). *Budget (2.5 k€) is requested for the purchase of B-precursor gas for the BDD electrode synthesis (~1 k€), enzymes, chemicals, and gold electrodes (~1.5 k€), respectively.*



COMPLEMENTARITY AND SYNERGY: There is no current on-going collaboration between these two PIs that met at the 2018 Biodate. It is an interdisciplinary project with an innovative approach to contribute to biosensing, spanning enzymology, surface chemistry, materials chemistry.

- Dr Ivan Buijnsters (3mE) is an expert on engineering BDD electrodes. He implements novel design and manufacturing approaches, such as: printing, template-based growth, and laser scribing.

- **Dr Duncan McMillan** (**BT**) is a Microbial Physiologist/Biochemist who has biophysical skills in surface chemistry techniques such as QCM-D, bioelectrochemistry and thiol surface modification.

LONG-TERM VISION: The initial phase of this MSc project (~1 year) will be continued by a new research line and long-term collaboration on building high-performance biosensors, and we will apply for external funding at both national and international level. Follow-up proposals to attract PhD & PD in the fields of biosensing, C-based nanomaterials, and enzymology are planned within NWO's domains ENW and ZonW, the framework of the Open Technology Programme (TTW) as well as upcoming H2020 calls, e.g. DT-NMBP-03-2019 (Open Innovation Test Beds for nano-enabled surfaces and membranes).

BioDate 2018 MSc project proposal

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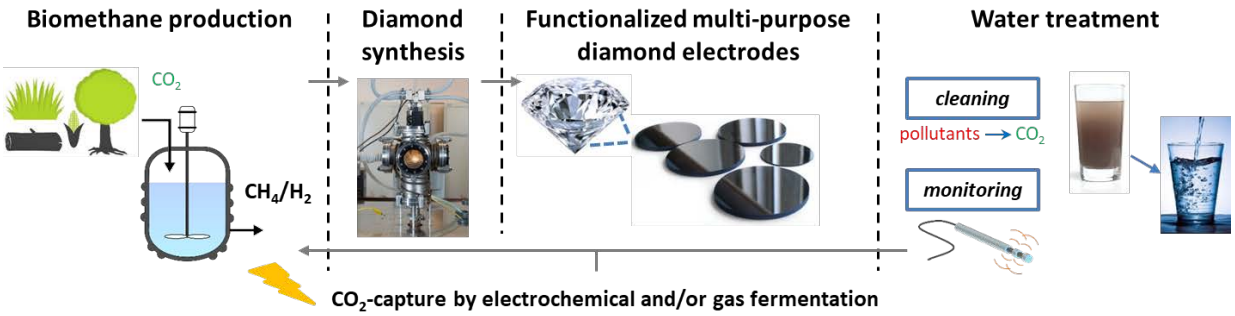
Expertise for the Project: diamond synthesis, materials engineering	Expertise for the Project: biomethane production, water treatment

Graphical abstract



Title: Production of diamond electrodes from biomethane for efficient drinking water treatment

Aim: We aim to demonstrate a novel innovative and truly unique resource recovery concept in which biogas produced from organic waste (-water) is converted into thin-film diamond electrodes that are highly efficient for micropollutant removal from drinking water by electrocatalytic processes.

Scientific background: Diamond, a carbon allotrope, is often considered the ultimate engineering material since it combines superior properties like extreme hardness, chemical robustness and highest thermal conductivity. When boron-doped, it also becomes a very good electrical conductor. Chemical vapor deposition, a thin-film growth process using gas mixtures of primarily methane and hydrogen, is able to produce the diamond electrodes in many forms and shapes and thus opening up a wide array of novel applications, which were not realistic using "mined" diamond.

Impact: Latest research demonstrates that the anodic treatment of B-doped diamond (BDD) leads to the direct and efficient generation of highly reactive hydroxyl and oxyl radicals from electro-oxidation reactions, which exhibit a strong oxidizing power to decompose recalcitrant organic components in water treatment applications (Chem. Comm. 53 (2017) 1338), which is one of the most pressing topics in both the potable and wastewater domain. Interestingly, diamond growth and carbon materials synthesis in general (e.g. fibers and nanotubes) are carbon capture and utilisation (CCU) technologies and could potentially serve as a carbon sink. By using biogas instead of natural gas, carbon will be sourced from the short carbon cycle and can be considered "carbon-negative". Moreover, anaerobic digesters, as decentralized sources of "industrial" CH_4, could reduce solid carbon synthesis dependency on a centralized and politically sensitive natural gas network. Digesters producing less than 100 $m^3\ h^{-1}$ were considered small from a biogas upgrading point of view (Lindeboom, 2014, ISBN: 978-94-6173860-8), but are considerably larger than the industrial scale diamond synthesis reactors using max. few L min^{-1}. The Power-to-Gas concept could be used to tune the biogas mixture to a CH_4/H_2 mixture suitable for diamond synthesis using decentralized renewable energy sources. It is noteworthy that absence of N_2 in biogas can be considered an advantage over "Slochteren" natural gas as it would simplify gas upgrading for diamond production.

Complementarity: At PME-3mE, Ivan Buijnsters initiated a novel line of research on diamond engineering and the development of multi-purpose diamond electrodes for use in electrochemistry and catalysis is at the core of his tenure-track research. He aims to exploit the huge potential of BDD in a wide range of applications, incl. electrochemical sensing and water treatment and monitoring. At WM-CEG, Ralph Lindeboom aims to recover resources from wastewater in decentralized settings like Outer-Space and arid regions. His high pressure bioreactor research shows that biogas quality without external upgrading can exceed natural gas quality. But in order to recover the most abundant resource in wastewater, potable water, he co-operates closely with his drinking water colleagues, like Yasmina Doekhi-Bennani, who compared several photoelectrocatalysts for removal of recalcitrant trace compounds in potable water production. BDD came out on top.

Activities in MSc project: We propose a joint MSc project that focuses on: 1) the feasibility of biomethane as a precursor gas for the production of diamond electrodes, and 2) the functionalization and use of such electrodes for potable water production (i.e. photoelectrocatalysis, advanced oxidation, etc.) will be explored. The budget of 2.5 k€ will be allocated to materials and chemical analyses needed to construct a prototype diamond growth setup suitable for biomethane.

Long-term vision: We foresee a long-term collaboration on the use of diamond electrodes for water treatment. The outcomes of the MSc research will be used to jointly apply for Dutch and international grant schemes and funds in collaboration with industrial partners and water utilities (Mintres BV, PWNT, Everest Coatings, Magneto BV).

BioDate 2018 MSc project proposal

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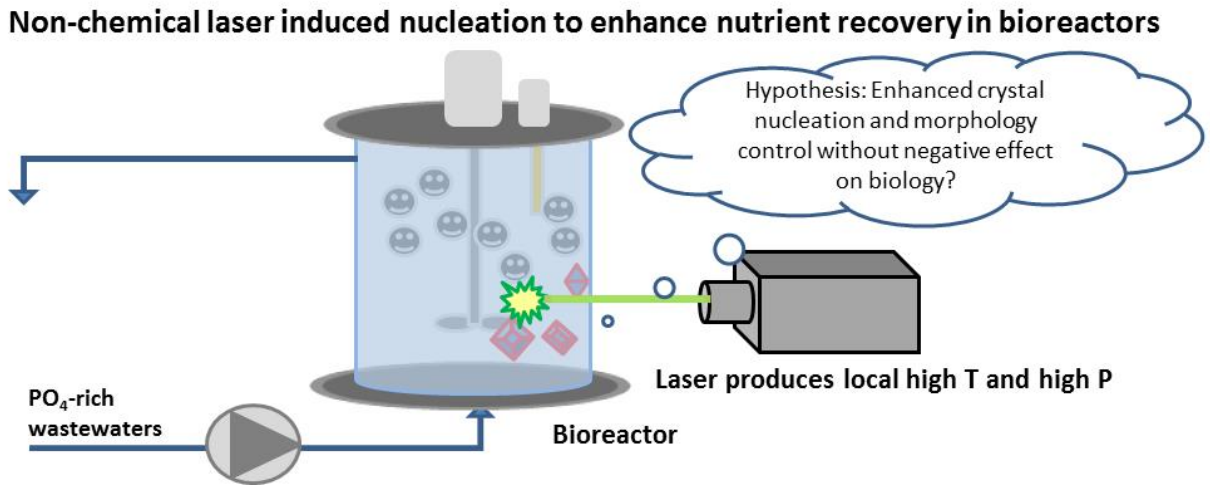
Expertise for the Project: Laser induced Crystallisation, Physics, Modelling	Expertise for the Project: Watertreatment, Biotechnology & Biocrystallisation

GRAPHICAL ABSTRACT



TITLE: Non-photochemical laser induced nucleation to enhance nutrient recovery in bioreactors

Aim: The aim of this master project is to explore the possibility of using non-photochemical laser induced nucleation (NPLIN) to remove components that are otherwise difficult to crystallize/separate in bioreactors. Particular emphasis will be placed on water purification and integration of NPLIN to existing bioreactor designs.

Scientific background: Almost two decades ago, NPLIN was discovered accidentally by Garetz et al[26]. while studying the nonlinear optical properties of supersaturated aqueous solutions of urea. It was reported that nanosecond pulses of near infrared light (1064 nm) could drastically reduce the induction time of urea crystals --from weeks to minutes-- and they reported that the alignment of the crystal is controlled through polarization of the incident beam. The transparency of the supersaturated solutions to the incident light distinguishes it from the well-understood phenomena of precipitation by the photochemical reactions. Garetz et al. proposed that nucleation is induced by the optical Kerr effect (OKE) which would induce an orientation of the solute molecules in the solution due to the electric field of the laser beam. Others have criticized OKE hypothesis and proposed cavitation based hypothesis[1]. In a nut shell, the exact mechanism of NPLIN is still under debate while plethora of application are ripe for picking[2].

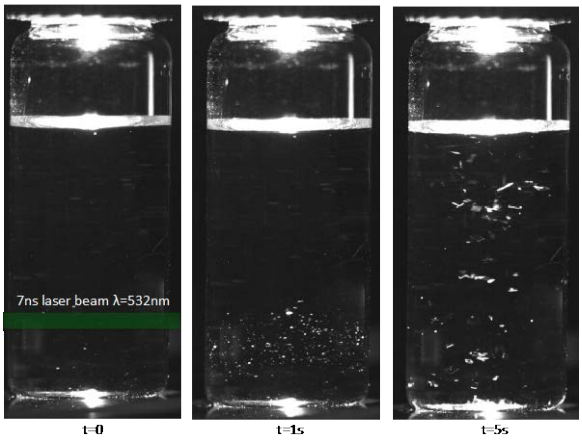


Figure 1 NPLIN in action. Supersaturated KCl solutions are exposed at t=0 and crystallization is observed seconds later. Experiments in Eral lab by master student S.Dignhra

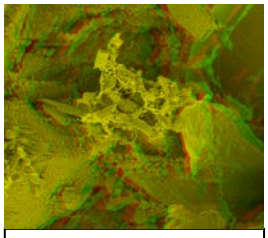


Figure 2: 3D HR-FESEM picture of close microbe mineral interaction (to be viewed with red/ green glasses)[4]

Impact: Within wastewater treatment, biocrystallisation is a new trend that should allow for the specific biorecovery of metals[3] and nutrients, like struvite or TriCalciumPhosphate. The production of pure crystals is however very challenging, due to the complex ion matrix, in which multiple crystals are simultaneously supersaturated and due to the long crystal induction time that provides microbes the opportunity to entrap crystallized products in a biofilm (Figure 2). We expect that by using NPLIN, we can accelerate the nucleation kinetics, extend our control over the crystal size, purity and morphology and thus improve crystal harvesting. We expect this to open up new pathways for crystal/ resource recovery from wastewater that move beyond current state-of-the-art techniques in separation efficiency and product quality.

Complementarity of researchers and disciplines: H.B. Eral brings in the expertise in NPLIN and design of separation processes. R.E.F. Lindeboom adds his expertise in ion speciation (phreeqC),wastewater treatment and bioreactors. The laser infrastructure currently available in P&E and operational bioreactor setups in CEG are available. This unique combination of expertise, equipment and know-how is essential to successfully supervise this multidisciplinary MSc- project.

Feasibility of proposed MSc-research: Initially aqueous solutions containing calcium and phosphate ions will be used as model waste water. Systematically we will increase the degree of complexity to reach realistic bioreactor broth. We will measure the induction time for biomineralization as a function of supersaturation and bacterial content. The MSc outcomes will be used as input for joint national and international grant applications.

[1] R.Kacker et al Crystal Growth & Design 18 (1), 312-317 (2017) , [2] F. Watson, K. Wynne Nature Chemistry 10, 506–510 (2018), [3] Hennebel, T. et al., New biotechnology 32, 121-127 (2015) [4] Lindeboom, 2014, ISBN: 978-94-6173860-8

BioDate 2018 MSc project proposal

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Fluid manipulation

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Guidelines

- Team of at least two PIs from different faculties / departments
- The PIs participated to the BioDATE and have no active collaboration
- Interdisciplinary project
- Feasible for students from at least one education program
- Max 2 proposal for every PI

Eligible projects are evaluated by a review committee for:

- Scientific quality
- Complementarity
- Feasibility
- Impact

See evaluation criteria rubric for more specific information

TITLE: Unravelling the dynamics of DNA transport through single electro-pore using micro-fluidic AFM

Summary:
Safe and efficient delivery of DNAs into a living cell is a critical step for DNA vaccination, and gene therapy for cancer treatment. So far, the gene electro-transfer has been proven to be one of the safest, best and simple non-viral methods of delivery, however it suffers from poor transfection efficiency, random uptake and with low cell viability. Due to our lack of knowledge on the mechanism of DNA electrotransfer, and basic physical process responsible for the electropermeablization of cellular membranes, gene electro-transfer is crude and optimized based on trial and error approach.

This proposal aims to unravel the dynamics of biophysical processes involved in DNA electrotransfer using an artificial cell model. We will develop 1. A novel setup to simultaneously monitor biophysical processes and DNA transport during electroporation, 2. Quantify the electroporation dynamics (surface tension, pore size, rate of pore formation, rate of DNA molecules transported), 3. Develop methods to controllably create single electropores in lipid bilayers. Beyond the DNA electroporation, the knowledge developed by this unique technology can lay foundation to understand the transfection of variety of nanoscale components across the cell membrane.

Aim:
The main aim of this proposal is *to create a novel methodology based on a microfluidic AFM to unravel the electro-transfer mechanism of DNA into the cell by decoupling active and passive transport phenomena*. We will create an artificial cell model system that emulates the subtleties of the real cell physiology, sufficiently controllable to monitor biophysical processes and DNA transport in a physiologically relevant environment.

Research Question:
Does the DNA transport during electroporation happen with brief pore formation or endocytotic process?

Novelty:
The novelty of the proposal lies in the creation of lipid bilayer sizes on-demand, generate controlled electro-pores, transport the chosen molecules (DNA in our case) and measure variety of biophysical parameters (surface tension, pore size, rate of pore formation, rate of DNA molecules transported) *in-situ*. Improving our knowledge of DNA transport inside the cell-like environment will have implications on our understanding of DNA transfection via non-viral method.

Approach and feasibility:
In this project will be to use hollow AFM probes for the first time in the electroporation studies. The technology to fabricate the hollow AFM probes already exists in Ghatkesar's group (TUD/3me). This unique probe enables the fluid to pass through the microfluidic channel of the cantilever and exit through the narrow aperture at the cantilever tip. A 150 microns long U-shaped hollow silicondioxide cantilever with 2.5 x 3.5 microns of channel cross-section and an aperture of about 1 micron in diameter is shown in Figure 1a and 1b. Initial experimental results on the flurorescene droplet dispensing in water (Figure 1c) and water droplet dispensing in oil (Figure 1d) is already achieved. The droplets are monitored through an inverted optical microscope while the cantilever is dispensing from the top side.

To monitor the transport of DNA (with different sizes) during/after electroporation, we will create an artificial cell membrane model by combining hollow AFM on a JPK instruments NanoWizard® instrument with CellHesion® module with confocal microscopy in Boukany's group (TUD/TNW) (Figure 1e). Fluorescently tagged DNA and lipid-bilayer surface area will be visualized with confocal microscope (CFM) from bottom, while the parameters, pore-current and membrane surface-tension, are monitored from the topside with force-sensitive AFM. The hollow probe is connected to a reservoir where the pressure (over pressure or under pressure) applied can be varied to control the droplet size generated near the aperture. The key advantages of using hollow AFM probes are to obtain droplets on demand. However, the challenge is not to obtain a droplet of desired size but to maintain the droplet at that size. The droplet size obtained with these probes is a delicate balance between the applied pressure to the channel reservoir and the Laplace pressure inside the droplet. If the Laplace pressure inside the droplet is higher than the applied pressure, the droplet will shrink and vice-versa. As the setup will be on an inverted microscope, we will monitor the droplet size image and use that as a feedback to control the pressure to maintain the

droplet at the desired size. Furthermore, the entire probe will be coated with surface assembled monolayer of suitable molecules to avoid droplets attaching to the probe surface and hence obtain as round droplets as possible.

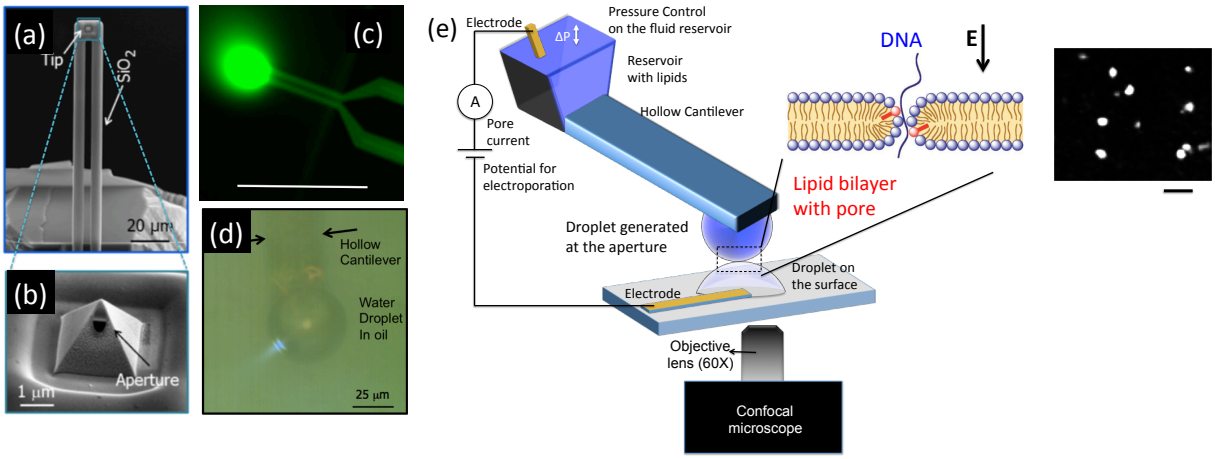

***Figure 1**: (a) Hollow AFM probe, b) Aperture near the apex of the tip c) A fluorescent image of an AFM tip dispensing a fluorescent dye droplet in water, d) A water droplet in oil near the probe tip aperture in oil and (e) The schematic of the planned set-up for investigation of DNA dynamics during/after electroporation. The inset at top right shows the individual DNAs (5 kbp) have been imaged under CFM. The scale bar is 3 μm.*

Multidisciplinary/complimentary expertise:
The proposal involves in applying the latest developments in the AFM probe technology to solve the mysteries in electroporation. The idea demands multidiscipline knowledge of the micro/nano fabrication methods, precision fluid control procedures, sensitive force measurement methods, biochemical protocols to emulate cell functionality in a lipid vesicle and of DNA transport properties. Ghatkesar and Boukany groups are perfectly complimentary with these expertise and together bring the needed knowledge to develop this unique methodology.

Student profile and time-plan:
This project fits in both engineering educational programs: The student (MSc, chemical engineering or mechanical engineering background) will fabricate a hollow AFM probe to create droplet interface lipid bilayer model to emulate cellular and sub-cellular membrane during DNA electro-transfer.

Time plan:
Literature review (2 months), microfluidic cantilever fabrication (1 month), AFM+confocal microscope experiments (4 months), development of biophysical model (2 months), reporting (1.5 months)

Next step:
The goal of the master project will be to generate the preliminary data. Eventually, both PIs will write a joint grant proposal (funding target: TTW or HTSM) to address the research question in detail through systematic studies by hiring PhD/Postdoc researchers.

Impact:
With the results obtained, we hope to solve the long-standing mystery of electroporation. We will understand the dynamics of the electroporation with quantitative information of various biophysical processes. Overall, this project paves the way for more safe and efficient DNA electro-transfer into cells leading for successful cancer treatment and gene expression. The methodologies that we will develop will not only be confined to DNA transport but can be extended to the transport phenomena studies of any molecule through electroporation. The artificial cell model that we develop will be made further better to emulate more aspects of the real cell functions. The next steps must be to bridge the gap between the insights afforded by these in vitro models, and the realities of electroporation in vivo.

BioDate 2018 MSc project proposal

Please submit your project proposal to Nienke van Bemmel: N.vanBemmel@tudelft.nl

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Department/Faculty: Bionanoscience
Expertise for the Project:
Protein biochemistry, cryo-EM, biological system

Researcher #2
Name: Carlas Smith
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Department/Faculty: 3mE
Expertise for the Project:
Super-resolution microscopy, single-molecule kinetics, image analysis

TITLE: **Cellular self-defense monitored one molecule at a time**

SUMMARY:

All mammalian cell types have the ability to deploy host factors for defense against invasive pathogens. This universal and ubiquitous system of cellular self-defense is known as cell-autonomous immunity. Immunity-associated GTPases (IAGs), are essential components of the cell-autonomous immunity pathway. Activation of IAGs unleashes their antimicrobial activity toward intracellular bacteria, viruses, and parasites. To evade recognition by innate immune surveillance pathways, many intracellular pathogens reside inside vacuolar niches permissive for microbial growth and survival. While compartmentalization provides attractive habitats, it also facilitates unique defense strategies. IAGs attack these vacuolar compartments through the orchestrated assembly into supramolecular complexes on the pathogen-containing vacuole membrane. Powered by GTP hydrolysis, these IAG complexes compromise integrity of the vacuolar compartment, promoting membrane permeabilization and pathogen exposure to downstream antimicrobial pathways. The Biomolecular Electron Nanoscopy Lab (Arjen Jakobi) studies the conformational and oligomeric changes underlying the mechanism of IAG-mediated membrane rupture in atomic detail using electron cryo-microscopy. Conversely, our current understanding of the kinetics and temporal hierarchy of functional IAG assembly on pathogen-associated vacuole membranes is very limited.

In this project, the candidate **student will establish a precisely controllable recombinant system for monitoring assembly hierarchy and conformational changes of supramolecular IAG assemblies** on unilamellar vesicle membranes for use in **Colocalization Single-Molecule Spectroscopy (CoSMoS)** with multi-wavelength total internal reflection fluorescence (TIRF) microscopy.

To enable kinetic analysis of IAG assembly on modelmembranes, the student will develop methods to specifically introduce fluorophores into individual IAG monomers (host: ***Arjen Jakobi Lab***). Protein labeling will be accomplished using homologous recombination to fuse either a SNAP (alkyl-guanine S-transferase) or an E.coli DHFR (dihydrofolate reductase) tag onto the N-terminus of IAGs. These tags will permit incorporating photostable organic dyes into monomer units of IAG assemblies. Alternatively, cysteine engineering will be used to introduce site-specific fluorescent labels. Integration of two or more orthogonal tags will allow for simultaneous monitoring of different sub-complex assemblies by CoSMoS.

Using the specifically labeled protein constructs, the student will set up a system for CoSMoS to establish the hierarchical assembly of supramolecular IAG complexes on immobilized lipid vesicles using single-molecule TIRF microscopy (host: ***Carlas Smith Lab***). The student will establish liposome immobilization in a fluidic flow channel for TIRF microscopy and co-localization spectroscopy. This set-up will form the basis for detailed studies into the kinetics, assembly hierarchy and stochiometry of IAG assemblies on lipid membranes.

The expertise from both host labs is highly complementary. The Arjen Jakobi lab brings in substantial experience with the biological system under investigation, recombinant protein expression and labeling as well as high-resolution electron cryo-microcoscopy and image processing of membrane remodeling complexes. The Carlas Smith lab provides expertise in single-molecule microscopy, co-localization spectroscopy, single-molecule kinetics and image analysis.

The project is suitable for master students of the Nanobiology or Applied Physics programs. For either of the two cohorts the project provides the opportunity to participate in research not commonly accessible in their respective study programs.

All required equipment is in place. Recombinant protein expression of IAGs and vesicle loading has been established in the Arjen Jakobi Lab. All optical microscopy instrumentation is available in the Carlas Smith Lab, as are computational workflows for CoSMoS data analysis. The awarded financial assistance will be used as a contribution to the purchase of fluorescent dyes for protein labeling and lipids for lipid vesicle formation.

Month 1-3 Arjen Jakobi Lab	**Month 4-7** Arjen Jakobi / Carlas Smith Labs	**Month 8-10** Carlas Smith Lab
- Protein labeling - Monitor ability of labeled proteins to form assemblies on membranes using electron microscopy	- Establish liposome immobilization in TIRF flow channel - Establish CoSMoS assay with two/three model IAGs - Data collection	- CoSMoS data analysis - Writing of report

The recombinant system and CoSMoS set-up established by the student in the course of this project will provide unique possibilities to study kinetics and hierarchy of complex assembly pathways of supramolecular complexes on lipid membranes. By simultaneously observing multiple species, it will provide the opportunity to distinguish assembly pathways relevant to activation from off-pathway processes and define the order and reversibility of on-pathway binding and dissociation steps. The established methodology should generate preliminary data for joint grant applications and provides the basis for a lasting collaboration. Detailed knowledge of the molecular mechanism underlying IAG-mediated membrane permeabilization may provide opportunities to design host-directed strategies to control infection by vacuole-resident intracellular pathogens.