KIND Synergy Proposal – Carolien Bastiaanssen

Applicant

Name: Carolien Bastiaanssen

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Most significant achievements

During my PhD, I developed an unprecedented high-throughput platform (named SPARXS) for single-molecule interaction measurements of millions of sequences in a single experiment (Severins *et al.* under second review at Science, Bastiaanssen *et al.* submitted to Nature Protocols). By combining single-molecule fluorescence microscopy with next-generation sequencing, this technique opens the exciting dimension of sequence space for the single-molecule field. I also extended the technique to RNA libraries and further improved it for the study of DNA-DNA and RNA-protein interactions (Bastiaanssen *et al.* two research articles, in preparation). A second focus of my PhD work was characterizing a newly discovered Argonaute protein. This was a highly multidisciplinary project in which labs with expertise in phylogenetics, structural biology, molecular biology and single-molecule biophysics joined forces (Bastiaanssen *et al.* accepted in Nature Communications).

Title of the project

KIND-SOFI (kinetic multiplexing with DNA probes for SOFI): Fast super-resolution imaging of multiple cellular targets

Kavli host groups

Kristin Grußmayer group (main, https://www.tudelft.nl/grussmayerlab) and Chirlmin Joo group (supporting, www.chirlmin.org), both Department of BioNanoScience

Abstract

Super-resolution microscopy was a breakthrough in the fluorescence microscopy field as it can resolve details below the diffraction limit. However, while cellular processes often involve interactions between many different molecules, the currently available super-resolution techniques are not well suited for fast simultaneous visualization of many components in a cell. To fill this gap, I will utilize the high-throughput platform that I recently developed for establishing a fast, versatile and highly multiplexed super-resolution technique: KIND-SOFI (kinetic multiplexing with DNA probes for SOFI). This technique will be perfectly suited to visualize many different components in a cellular context, and will thereby become a valuable tool in gaining a better understanding of the interplay between different molecules in complex cellular processes.

Description of the proposed research

Background / motivation / state-of-the-art

Super-resolution microscopy techniques have proven to be extremely valuable in resolving the fine details of cellular structures and processes. One family of super-resolution techniques is single-molecule localization microscopy (SMLM), which relies on the temporal separation of closely spaced emitters. This is achieved by for example using photoswitching dyes or transiently binding DNA probes. The latter is known as DNA-PAINT (DNA points accumulation for imaging in nanoscale topography). While a diverse range of techniques have been developed that beat the diffraction limit, many of them suffer from very specific fluorophore requirements, phototoxicity, complex sample preparation or long acquisition times. This prevents them from being more widely applied and makes it harder to multiplex them. The latter is essential to answer outstanding biological questions that often involve multiple molecular species.

Super-resolution optical fluctuation imaging (SOFI), expertise of the Grußmayer group, avoids many of the aforementioned disadvantages. It is therefore very well suited for super-resolution imaging in cells. This technique is based on the fact that the light emitted from each fluorophore is temporally and spatially correlated with itself and uncorrelated with that of others. By analyzing the stochastic fluctuations in pixel intensities with higher-order

statistics, SOFI provides a gain in resolution and enables the removal of background and out-of-focus light. Although the maximum achievable resolution with SOFI is lower than that of SMLM, its lower demands on the sample make SOFI applicable to a broader range of biological systems.

Multiplexing with SOFI can be achieved using spectrally distinct fluorophores and spectral cross-cumulant analysis can further increase the spectral sampling. Another approach is based on differences in blinking kinetics of reversibly switchable fluorescent proteins. Both approaches have been demonstrated for imaging of two or maximally three different cellular targets. However, often many more different molecular species are involved in cellular processes and there is a need for higher order multiplexing.

Aim

The aim of this project is to enable fast simultaneous super-resolution imaging of many cellular targets. To this end, I will develop KIND-SOFI: kinetic multiplexing with DNA probes for SOFI. It has already been shown separately that SOFI is compatible with short transiently binding DNA probes as used in DNA-PAINT and that with SOFI parameter estimation the per pixel on-time ratios of the emitters can be determined. While the programmability of DNA allows for the creation of a large probe set with distinct kinetics for kinetic multiplexing, this unique opportunity has not yet been exploited. In this project, I will demonstrate the capabilities of KIND-SOFI in cells and ultimately this technique will be perfectly suited to answer questions such as: 'How do coordinated interactions among various cytoskeletal proteins dynamically regulate cell shape and motility?' and 'What is the effect of different synaptic contexts on the localization and function of a neuronal protein of interest?'.

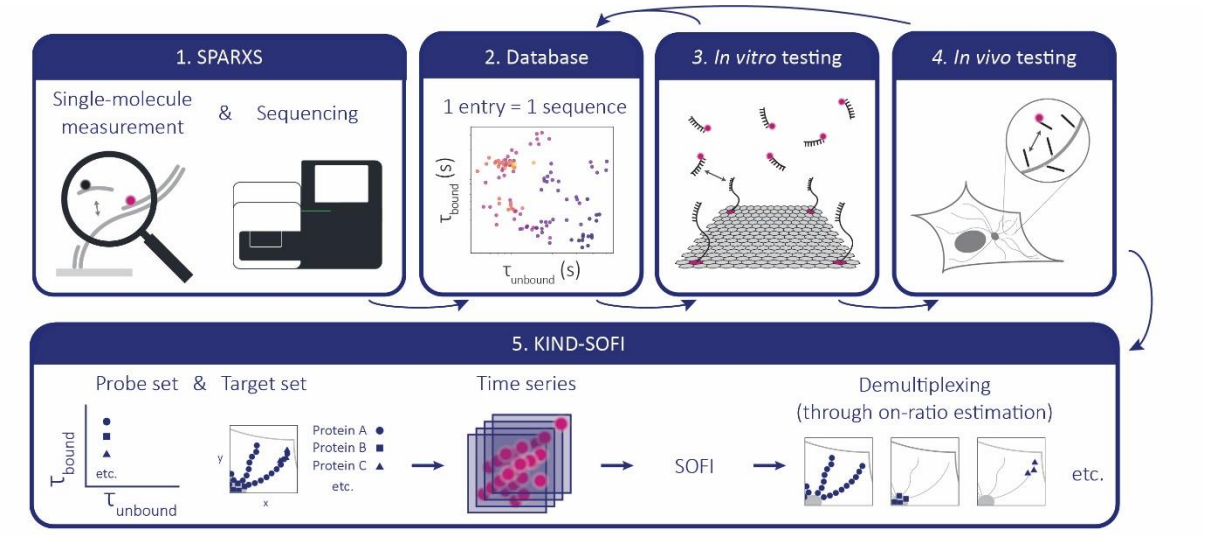


Figure 1: Approach for the development of KIND-SOFI. (1) A DNA hybridization SPARXS assay is performed on a library of thousands of DNA sequences. **(2)** The output of the SPARXS assay is a kinetics database for all sequences in the library. **(3)** Candidate sequences are selected for *in vitro* testing on DNA origami plates. **(4)** Promising candidates are tested *in vivo*. **(5)** The carefully composed probe set will be used for KIND-SOFI in cells, where multiple proteins will be imaged with a mix of probes exhibiting distinct kinetics. Through SOFI parameter estimation, a per pixel estimate of the on-time ratio can be obtained. This will be used to demultiplex the data. During stages 3-5 of the project, the database will continue to be consulted to optimize the probe set.

Approach

During my PhD, I established a high-throughput SPARXS assay to determine the kinetics of a library of short DNA probes in a single experiment and I have demonstrated that this can be used to for example find a faster probe for *in vitro* super-resolution imaging of DNA origami with DNA-PAINT (Bastiaanssen et al, in preparation).

In this Synergy project, I will utilize my DNA hybridization SPARXS assay and the resulting kinetics database to find a set of probes that allow kinetically multiplexed SOFI (**Figure 1**). I already have a dataset for 128 sequences as a starting point and am working on a dataset of 16,384 sequences. Additionally, for this project I plan to perform

the assay with a library of shorter binding probes as these might be more suitable for SOFI. Top candidates identified from the database will first be fully characterized in a controlled environment, for example on DNA origami. Additionally, this will enable testing of the analysis workflow. This part of the project will be performed in the Joo group with input from the Grußmayer group on SOFI analysis and on which kinetics to look for. The estimate is that this part of the project will take approximately 3 months to complete.

Next, promising sets of probes will be tested for cellular use by *in vivo* DNA-PAINT imaging before being used to set-up KIND-SOFI in cells. I will image well known reference targets such as microtubules, vimentin, mitochondria, nuclear membrane, etc. to establish the method. The Grußmayer group already has experience with labeling and imaging of these structures. Many different targets will be labeled with different primary antibodies that were pre-incubated with probe-conjugated secondary nanobodies. Secondary nanobodies and the procedure for coupling of DNA strands are already established. This core part of the project will be performed in the Grußmayer group and is expected to take approximately 6 months. Ran Huo in the Grußmayer group will serve as the day-to-day technical advisor throughout this phase of the project.

During the second part of the project, I will go back and forth between the SPARXS database and cell imaging as the probes might behave differently in a cellular context or we might realize that we are looking for slightly different kinetics than we thought. The synergy created by this project will help me to quickly and smoothly switch between the techniques used in both groups and will help with a timely execution of the project.

Host environment

The Grußmayer group specializes in developing cutting-edge super-resolution microscopy tools and has extensive experience with cell imaging. They can provide all the consumables, expertise, and microscopes necessary for cell and super-resolution imaging. This includes cameras that allow very fast imaging and event-based cameras, which will be crucial for KIND-SOFI and are not available in the Joo group or the Kavli Nanolab Imaging Centre. Combined with the novel SPARXS technology developed by the Joo group, all infrastructure and expertise needed for this project are readily available. Additionally, the pre-existing collaborations and networks of both groups offer numerous opportunities for applying the newly developed technology to address crucial biological questions.

Benefit to the career of the fellow

After my PhD in biophysics, which involved extensive use of *in vitro* single-molecule fluorescence microscopy, I want to transition to single-molecule imaging in cells. While *in vitro* approaches excel at revealing molecular mechanisms in detail, studying the same processes in their native cellular context enables us to better understand how they are regulated in a highly heterogeneous and dynamic environment. I realize that single-molecule imaging in cells brings new challenges. This Synergy Fellowship will enable me to master the skills necessary for cell imaging and it will also add super-resolution techniques to my toolbox. Besides the technical skills, it will also help me to expand my network from the biophysics and Argonaute community to the cell imaging and super-resolution microscopy communities. Thus, by enabling me to diversify my skills and giving me the opportunity to build a more extensive network, the Synergy Fellowship will provide me with an excellent basis to develop my own line of research and establish my own group. Using the results from this project, I will apply for a Veni grant in 2025/2026.

Benefit to the synergy of KIND

This project will be the first collaboration between the Grußmayer and Joo groups. By bringing together the super-resolution and cell imaging expertise of the Grußmayer group and the SPARXS technology from the Joo group, a powerful multiplex technique will be developed which will enable the use of SOFI to study a wider range of biological questions. This will provide better solutions for already ongoing collaborations with for example the Koenderink and Meijer groups at TU Delft and the Kanaar group at Erasmus MC. Additionally, this will strengthen the position of KIND in the forefront of the super-resolution microscopy field, opening up many opportunities for further collaboration with other groups, such as the Tanenbaum group, and also outside of KIND based on this technology. Besides the direct benefits relating to KIND-SOFI, this first connection between the Grußmayer and Joo groups is likely to be the spark for more collaborations where single-molecule biophysics and cellular super-resolution imaging are combined.