KIND Synergy Program − Research Proposal

1. Applicant

Dr. Marcos Arribas Perez, Postdoctoral researcher (Gijsje Koenderink group, Bionanoscience Department, TUD)

During my PhD at the University of Leeds, I studied the interaction between engineered nanoparticles (NPs) and biomimetic lipid membranes as an early indicator of potential nanotoxicity. I demonstrated that conditions such as the ionic strength or the presence of biomolecules in the medium have a strong impact on how NPs affect the biophysical properties of the membranes [1, 2]. Additionally, I discovered that silica NPs are able to drive fusion of lipid vesicles [3], an approach that can be used to create vesicles with complex lipid compositions by merging simpler membranes [4].

Driven by my interest in the interplay between the cell membrane and the cytoskeleton and aiming to direct my career to this topic I joined the Koenderink lab as a postdoc. Here, I have been investigating the actin cytoskeleton reconstituted within cell-sized vesicles. In collaboration with a PhD student, I successfully developed a robust new method to fabricate vesicles with an actin cortex and was able to demonstrate that this cortex actively deforms the vesicles by nonequilibrium polymerization forces [5, 6].

2. Title of the project

Anillin-mediated crosstalk between actin and microtubules in confined cell-like spaces

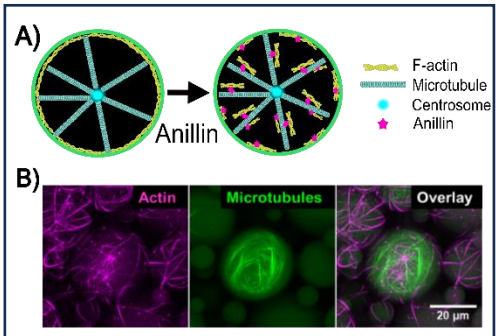
3. Kavli Host Groups

Gijsje Koenderink (BN) Link to GK lab website

Marileen Dogterom (BN) Link to MD lab website

4. Abstract

Cytokinesis is an essential cellular process that leads to the amazingly accurate division of a cell into two daughter cells of equal size. Evidence from cell biology indicates that this precision is achieved by coordination of the actin cytoskeleton that splits the cell and the microtubule cytoskeleton that segregates the chromosomes. Recently, anillin was proposed as a master regulator since it can directly crosslink the two cytoskeletal systems while at the same time scaffolding cytokinesis signalling cascades. However, proving this hypothesis and identifying the biophysical mechanisms behind precise midway cleavage is very challenging due to the high complexity of cells. Here, I propose a bottom-up synthetic biology approach to reconstitute the anillin-mediated crosstalk between actin and microtubules within a cell-like confined space. For this purpose, state-of-the-art microfluidic technology will be used to generate cell-sized emulsion droplets encapsulating the proteins of interest. By employing quantitative fluorescence microscopy I will directly investigate whether and how the direct contact between microtubules and actin mediated by anillin can pattern actin cortex structure and activity. This will provide insights into anillin's role as a key orchestrator in the coordination between actin and microtubules during cell division.



A) Hypothesis of the effect of anillin crosslinking in the organization of actin and microtubules within an emulsion droplet. B) Microscopy image of a droplet encapsulating microtubules and actin bundles (Preliminary experiment made in collaboration with Yash Jawale, Dogterom lab)

5. Description of the proposed research

Background and motivation

Cytokinesis, the final stage of cell division by which a cell splits in two, generating two exact copies of itself, is one of the most essential and intriguing processes of life. Central to this process is the cytoskeleton, a network of dynamic filaments made of different proteins. In animal cells, cell biology studies have shown that two main cytoskeletal components, microtubules and actin filaments (F-actin), work together to ensure the successful completion of cytokinesis. Microtubules align and bundle at the centre of the cell and recruit proteins that start a signalling pathway that reorganizes the actin cortex underneath the cell membrane, causing actin to localize in the middle where it then constricts the cell [7]. It is believed that direct crosslinking between microtubules and cortical actin is needed to stabilize the division plane. A candidate crosslinker protein is anillin, which is considered the master regulator of cytokinesis. During cytokinesis, anillin interacts with F-actin and actin-binding proteins (the actin-nucleator protein formin, its activator RhoA, and myosin 2 motors) as well as with proteins associated to microtubules at the mitotic spindle such as Ect2 [8]. Moreover, anillin has been seen to colocalize with astral microtubules. Based on these observations, it has been proposed that astral microtubules can remove anillin from the actin cortex at the poles of the cell, which in turns reduces the myosin concentration in those regions - limiting the actomyosin contraction to the equatorial cortex [9]. However, the complex cellular environment makes it difficult to

disentangle the biochemical and biophysical mechanisms that ensure precise mid-cell positioning of the cleavage plane. Recent *in vitro* work in the Dogterom lab has shown that anillin can act as an actin-microtubule crosslinker since it can directly bind microtubules and recruit F-actin to the microtubule lattice [10]. At the same time, in the last two years in the Koenderink lab I have been able to reconstitute realistic actin cortices and networks within cell-sized vesicles [5, 6]. In this project I will combine these advances and for the first time establish a synthetic cell model system for cytokinesis based on droplets containing an actin cytoskeleton and artificial microtubule spindle interacting *via* anillin.

Aim and approach

The aim of this project is to understand the mechanism by which anillin-mediated crosstalk between actin and microtubules controls cytokinesis by reconstituting a minimal synthetic cell model system. The experimental system will consist of water-in-oil emulsion droplets stabilized by a phospholipid monolayer and the proteins of interest encapsulated within them.

The **first objective** is to develop a pioneering method to co-encapsulate dynamic microtubules and lipid-bound F-actin inside the lipid droplets (5 months). Here I will build on my expertise rebuilding actin cortices inside lipid droplets and vesicles. I found in preliminary experiments that adding dynamic microtubule asters to the system is challenging. To address this challenge, I will use state-of-the-art microfluidics to generate emulsion droplets and explore different experimental conditions including, protein concentration, crowding agents and actin-membrane binding strategies. For the latter, I will use two different approaches: A) biotin-streptavidin binding by including biotinylated lipids and actin in combination with streptavidin proteins. B) F-actin nucleation at the membrane to create a more biomimetic actin cortex. For this I will use the actin nucleator formin mDia1, with a poly-Histidine tag that binds to lipids with a headgroup decorated with nitrilotriacetic acid (NTA)-nickel. In parallel, I will perform experiments where only one of the cytoskeletal systems is encapsulated together with anillin inside the droplets. This will serve as a control to confirm that anillin binds to both cytoskeleton components in confinement and to observe its effect on the organisation of the different filaments separately. All the protein components needed for the reconstituted system are purified in-house by technicians in the Koenderink and Dogterom labs, and confocal microscopes with complementary capabilities for quantitative imaging are also made available by both labs. In the **second phase** of the project (3months) I will for the first time build synthetic cell systems where microtubules and actin filaments interact *via* anillin crosslinking. This will allow me to assess if anillin-mediated crosslink affects the spatial organization and activity of the actin cortex due to the removal of F-actin and other components of the cortex by the microtubules, as it was observed to happen in vitro in non-confined conditions. Finally, I will evaluate whether the potential reorganization of the actin cortex derived from the anillin-driven crosslinking to microtubules can lead to a symmetry breaking with regions of the droplet showing more accumulation of cortical actin than others as proposed to happen in cells.

6. Host environment

The Kavli institute offers an ideal and unique opportunity for me to combine world-leading expertise in soft matter physics of actin (Koenderink group) and in biochemistry and biophysics of the microtubule cytoskeleton (Dogterom group). This complementary expertise together with the state-of-the-art imaging and nanofabrication equipment provided by both labs make this an excellent environment to perform the project proposed here.

7. Benefit to the career fellow

The KIND project represents an excellent opportunity to start building my future career. My long-term aim is to establish my own group. In order to start building up my own research line, my first step is to initiate this seed project, and use the results as a strong basis to write a proposal for a Marie Curie Postdoctoral fellowship with Professor Thomas Surrey in the Centre for Genomic Regulation (CRG) in Barcelona. My ambition is to study the signalling pathway between the actin cortex and microtubules during cytokinesis, leveraging the combined expertise of the Koenderink, the Dogterom and the Surrey labs.

8. Benefit to the Synergy of KIND

The Dogterom and Koenderink groups have previously collaborated on studies centred around single molecules facilitating interactions between actin and microtubules. This project open a new collaborative research topic towards building synthetic cells where different cytoskeletal components work together to recreate cellular processes in controlled environments. This initiative aims to enhance synthetic cell research within the Bionanoscience department. Furthermore, the benefit of this project will expand beyond KIND to an international collaboration with Prof. Thomas Surrey in the CRG in Barcelona.

References

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