# KIND Synergy Program — Research Proposal

**1. Applicant and 3 most significant publications:** Dr. Felix Frey, postdoctoral researcher, Idema lab, BN.

- **F. Frey** et al., *Eden growth models for flat clathrin lattices with vacancies*, New J. Phys. 22, 073043 (2020).
- **F. Frey** et al., *Stochastic dynamics of nanoparticle and virus uptake*, Phys. Rev. Lett. 122, 088102 (2019).
- D. Bucher*, **F. Frey*** et al., *Clathrin-adaptor ratio and membrane tension regulate the flat-to-curved transition of the clathrin coat during endocytosis*, Nat. Commun. 9, 1109 (2018). *equal contributions

**2. Title of the project:** Mutual control: how filament networks and membranes shape each other.

**3. Kavli faculty members that closely support this proposal:**

- Dr. Timon Idema, Department of Bionanoscience, Kavli Institute of NanoScience, TU Delft. Webpage: https://idemalab.tudelft.nl/index.html
- Prof. Dr. Gijsje Koenderink, Department of Bionanoscience, Kavli Institute of NanoScience, TU Delft. Webpage: https://www.tudelft.nl/en/faculty-of-applied-sciences/about-faculty/departments/bionanoscience/research/research-labs/koenderink-lab/

**4. Abstract:** The ability of cells to crawl and divide depends on their capacity to generate forces. The cytoskeleton, the cell's internal scaffold, produces forces which, when exerted on the cell membrane are translated into shape changes. Division of human cells requires membrane constriction by cytoskeletal septin filaments, but how exactly this causes the membrane to deform is still unclear. In this project, we will bring the experimental Koenderink group and the theoretical Idema group together to investigate the complex interactions between septins and the membrane. In particular, we will focus on the open question of how membranes control septin network assembly during self-assembly, which is challenging to analyze experimentally and thus requires modeling. By combining experiments, computer simulations, and analytical models, we will develop a new line of research in the largely unexplored field of coupled polymer–membrane composites, as filament networks and membranes were previously studied separately from each other. Our project will clarify how membranes control the assembly of septin networks and how their interaction leads to surface deformations, with the ambition to set up design rules to engineer cell division in a synthetic cell.

**5. Benefit to the synergy of KIND:** First, the collaboration will establish a new line of research at KIND that combines experiments, simulations, and theoretical modeling to investigate how septin filaments organize on membranes and thereby drive cell division, which is yet an unexplored area of biological research. The project also breaks new ground in biophysics of broad interest, since the studied filament networks and membranes are coupled into a composite material with new emergent properties. Second, the proposed project will initiate the first collaboration between the Koenderink and the Idema group, both working on soft matter and biophysics. Third, the project will stimulate discussions and thus be a springboard for future collaborations and joint grant applications between the Koenderink and the Idema group. Fourth, one motivation for the project is to understand the principles underlying cell division, similar to the ongoing BaSyC research initiative at KIND, aiming to build a synthetic cell. Because of the common interest to generate design rules for cell division, the project will thus expand and complement other research activities and promote synergies within KIND.

**6. Research proposal — background and state of the art:** In order to move and divide, cells need to adapt their shape. The necessary forces are exerted by the cytoskeleton, a network of filamentous proteins, and act on and through the cell membrane, a fluid lipid bilayer. As human cells divide, cytoskeletal septin filaments constrict the membrane (Figure 1a), but how these filaments deform the membrane is unclear. In particular, the complexity of the apparatus driving cell division has made it difficult to dissect the molecular mechanisms that are at play[1], such that a combined effort of new experiments and modeling is required to solve this question.

Septin filaments are non-polar and built from heteromers. They can assemble in solution and on charged surfaces like lipid membranes, where they form higher-order structures such as paired filaments, bundles, or rings[2]. However, the precise mechanisms that mediate the interactions between individual septins and between septins and the membrane are still elusive. Septin assembly is known to be curvature sensitive[3], but the mechanism is also unknown. Besides the biological relevance, the subject is also intriguing from a physical perspective, because it connects self-assembly to 2D surfaces, a topic that is usually studied in the 3D bulk. While a particularly well-studied example is the self-assembly of virus capsids in 3D[4], one of the examples in which self-assembly occurs on 2D membranes is the formation of clathrin coats on membranes during clathrin-mediated endocytosis[5]. Because of the templated geometry of the clathrin triskelia, however, the target coat structures have limited variability. The self-assembly of septin filaments is therefore different in many ways,

[1] J. Marquardt et al., Cytoskeleton, 2019
[2] A. Szuba, PhD thesis, Koenderink lab, 2019
[3] A. Beber et al., Nat. Commun., 2019 and K. S. Cannon et al., J. Cell Biol., 2019
[4] J. D. Perlmutter and M. F. Hagan, Annu. Rev. Phys. Chem., 2015
[5] M. Kaksonen and A. Roux, Nat. Rev. Mol. Cell Biol., 2018

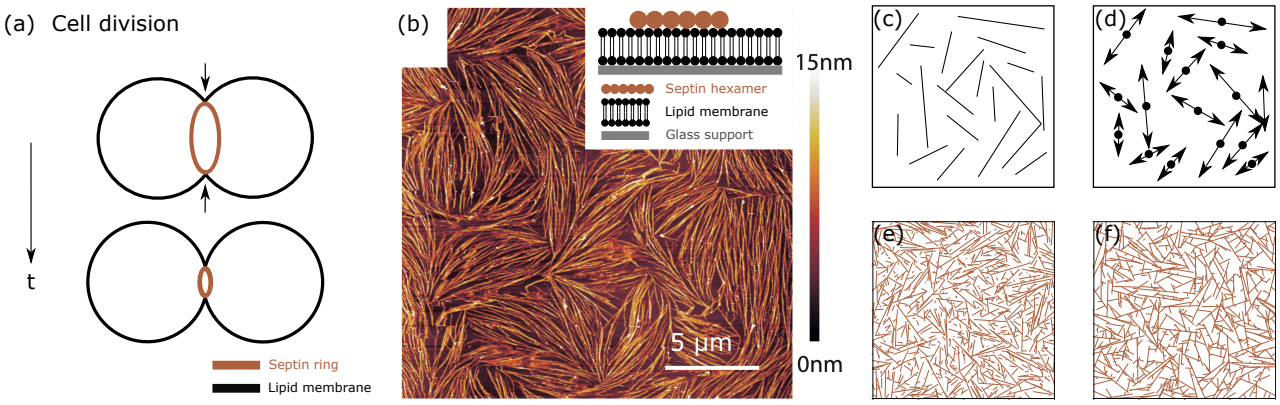



**Figure 1:** Motivation and preliminary results of the proposed project combining experiments, simulations, and modeling. (a) Schematic of the septin contractile ring that constricts the membrane during cell division. (b) AFM imaging shows bundled septin filaments and arrays on a lipid membrane from the top (color: heightprofile). Courtesy of A. Szuba, Koenderink lab. Inset: schematic of the experimental assay from the side. (c+d) Schematic of the mikado and snake model for septin filament assembly in solution and on the membrane. (e+f) Simulation snapshots of the mikado and the snake model.

since there is much more flexibility on how single filaments can grow and interact with each other and with the membrane. Moreover, septins can interact with other cytoskeletal networks that drive membrane deformations that potentially lead to feedback between the assembly and the deformation process. Taken together, the biological relevance, the broad physical character, and the novelty of the subject render the self-assembly of septin filament networks on 2D membranes a rich and very exciting field of research with high potential impact.

**Aim and approach:** In this project we will investigate the self-assembly and interactions of septin filaments on lipid membranes by joining forces between the experimental Koenderink and the theoretical Idema group. We will combine experiments, performed by Gerard Castro Linares, PhD student in the Koenderink group, and modeling performed by myself. The project consists of two phases. First, we will study the assembly of septin filaments on 2D membranes and their organization in multilayered stacks. Second, we will investigate how septin networks can interact with an active actin network, that can deform the membrane out of the plane.

In the first phase (8 months), we want to identify the nature and mechanisms by which human septins form ordered structures on flat lipid membranes. In the initial experiments, septins in solution are brought into contact with a glass-supported lipid bilayer, where, using atomic force microscopy (AFM), the assembly process is monitored (Figure 1b). The assembled filaments form structured bundles and arrays on the membrane. To understand the mechanistic role of the membrane in the assembly process, we first want to determine whether filament assembly begins in solution or on the surface, a question that is challenging to analyze experimentally and thus requires theoretical modeling. In the preliminary modeling work we consider two scenarios: In the mikado model we assume that septin filaments preassemble in solution and only arrange themselves on the membrane (Figure 1c), while in the snake model we suppose that septin filaments nucleate and grow only on the membrane (Figure 1d). Our preliminary results show that the simulated assembled structures differ in the resulting filament lengths and in structural network properties such as connectivity and order (Figure 1e and f). In the proposed project, we now want to systematically compare the simulation results with the AFM images by analyzing filament lengths, connectivity, and order to determine the role of the membrane during the assembly process. Using our modeling, we want to predict the network properties that will enable us to benchmark our modeling and to distinguish between different mechanisms for assembly. Additionally, we will include the possibility that septin filaments can interact with neighboring filaments in both the x-y plane and the z-direction. As observed experimentally, the latter will lead to multi-layered filament stacks[6]. The first project phase will be completed within the funded time period with a draft for a joint publication of the Koenderink and Idema group.

In the second phase (12 months), we will determine how septin filaments interact with acto-myosin networks at the membrane, and how this leads to membrane deformations. To this end, we will develop new experiments and the corresponding theoretical framework. We may learn about the design rules and mechanisms that lead to membrane deformation during cell division, which has implications for current efforts to build artificial cells. The second project phase will be initiated within the funded time and will then be continued in parallel to my other projects within my regular postdoc position at the TU Delft as a joint project of the Koenderink group and myself as part of the Idema group.

**7. Infrastructure:** In general, the Koenderink and Idema group will provide an optimal environment to carry out the project. While the Koenderink group are leading experts in the experimental investigation of cytoskeletal networks and experienced in working with septins, the Idema group provides great expertise on modeling lipid membranes. Due to the physical proximity between the two groups, exchange of ideas will evolve continuously such that rapid progress can be expected. Apart from funding my postdoc position through the KIND Synergy Program, the running costs of the project will be covered by the Koenderink and Idema group.

[6] F. Jiao et al., Nat. Commun., 2020