
PhD project: **Terminal phases of red blood cell enucleation**

Laboratory: Dpt « Physics and Engineering for Living Systems »
Centre Interdisciplinaire de Nanoscience de Marseille (CINaM)
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Context. Erythropoiesis is the process of generating red blood cells (RBCs). In mammals, RBCs do not contain nuclei. The enucleation process is known to occur during RBC precursor cell exit from the bone marrow through extremely small pores to join the blood microcirculation. However, the physical mechanisms involved in enucleation are still unknown, because (i) it is difficult to fabricate an *in vitro* device that mimics the crowded environment of the bone marrow and the narrow gaps, and (ii) the force quantification is challenging due to the strong interactions between the cell and the surrounding fluid in the small gaps.

Project. **The project aims to elucidate the physical mechanisms at play in enucleation.** We focus on the role of the mechanical constraints in the bone marrow on the extrusion and detachment of the nucleus from the RBC precursor cell. The PhD student will design and fabricate a microfluidic device (Figure 1) consisting of a chamber (mimicking the bone marrow environment) connected via narrow gaps to a channel (mimicking a blood vessel). He/she will then perform microfluidic experiments with RBC precursors (extracted from mouse bone marrow) to determine the experimental parameters for efficient enucleation (chamber crowding, gap geometry and flow conditions for efficient nucleus extrusion and detachment). Once the experimental protocol is optimized, he/she will observe and quantify the cells/nuclei behavior by videomicroscopy. He/she will perform image analysis to extract features like extrusion rate, transit time through gaps, transit velocity, cell/nucleus deformation... The project will be done in collaboration with a computational scientist expert in multiscale modeling of cells (Z. Peng, Univ. Chicago at Illinois, USA) and a biologist expert in erythropoiesis (P. Ji, Northwestern Univ., Chicago, USA). Our data on cells from wild-type mice (normal cells) will be compared to similar data acquired in the biologist lab on cells from genetically modified mice (altered cell components to modulate the cell response in a controlled way). The experimental results will be confronted to predictions from the numerical model in which the mechanical and molecular parameters will be tuned. Our combined experimental and computational approach will allow us to decipher the internal and external forces that drive RBC enucleation. The work is primarily experimental, and simulations will be performed with the collaborator. The student will learn microfabrication techniques in the CINaM clean room facility, microscopy techniques, and image analysis. In the frame of the project the student will visit the collaborators once a year.

Expected profile. We are looking for a motivated candidate with a taste for experimentation. The student will have preferentially a mechanics/physics/biophysics background and should be keen to learn new technologies, with a strong interest towards biological questions. Experience in microscopy and/or biology is a plus. Good level in English speaking and writing is mandatory.

Funding. Grant from Doctoral School ED352

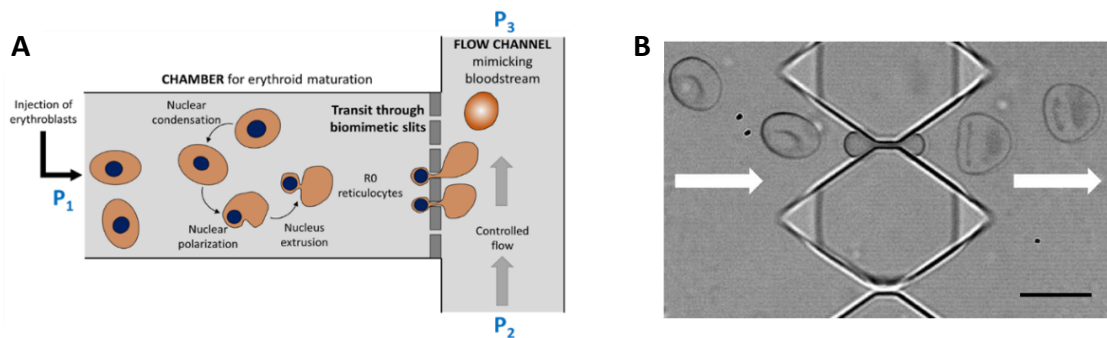


Figure 1. (A) The device will be designed and produced based on the CINA expertise acquired while developing a first device (B) mimicking the narrow slits in the spleen, an organ which filters RBCs [1,2]. (C) Microscopy observation of a deformable red blood cell passing through a biomimetic splenic slit ($0.95 \times 3 \times 4.7 \mu\text{m}^3$). The flow is indicated by the white arrows. Scale bar: $10 \mu\text{m}$.

Location. The PhD will be conducted within the Physics and Engineering for Living Systems (PIV) department of the Interdisciplinary Center for Nanoscience in Marseille. The laboratory is located on the Luminy campus in the heart of the Calanques Park.

Documents to provide:

- The candidate's CV
- A letter of motivation
- Two letters of recommendation
- Copies of diploma
- Transcript and academic honors

References

- [1] High aspect ratio sub-micrometer channels using wet etching: Application to the dynamics of red blood cell transiting through biomimetic splenic slits. P. Gambhire, S. Atwell, C. Iss, F. Bedu, I. Ozerov, C. Badens, E. Helfer, A. Viallat, and A. Charrier. *Small* 13, 1700967 (2017). <https://doi.org/10.1002/sml.201700967>.
- [2] Physical mechanisms of red blood cell splenic filtration. A. Moreau, F. Yaya, H. Lu, A. Surendranath, A. Charrier, B. Dehapiot, E. Helfer, A. Viallat, and Z. Peng. *Proc. Nat. Acad. Sci.* 120, e2300095120 (2023). <https://doi.org/10.1073/pnas.2300095120>.