Myosin modulated cellular biomechanics in the invasion process of the bacterium Shigella

Supervisor: Indicate the references of the person who will directly supervise the student’s project.
Name: Berret Jean-François
Phone: 01 57 27 61 47
E-mail: jean-francois.berret@univ-paris-diderot.fr

Host Laboratory: Indicate the references of the laboratory where the student will work
Affiliation: Université Paris-Diderot (Paris 7)
Lab Name: Laboratoire Matière et Systèmes Complexes
Address: UMR 7057 Université Paris-Diderot/CNRS, Batiment Condorcet, 10 rue Alice Domon et Léonie Duquet, F-75205 Paris Cedex 13

Co-Supervisors: Indicate the references of other researchers involved in the project supervision.
Name: Tran Van Nhieu, Guy
Phone: 01 44 27 14 89
E-mail: guy.tran-van-nhieu@college-de-France.fr
Affiliation: Center for Interdisciplinary Research in Biology, team: Intercellular communication and microbial infections
Address: Collège de France, INSERM 1050, 11 Place Berthelot 75005 Paris

Context and position of the proposal

Shigella bacterial invasion mechanism

Shigella is a bacterial pathogen responsible for bacillary dysentery in humans, a diarrheal disease predominant in developing countries. Shigella has the ability to invade epithelial cells from the colon (Fig. 1), and a key virulence factor to this process is a specialized secretion apparatus enabling the injection of bacterial effectors (i.e. proteins) into the host cells [1,2]. Shigella invades cells by inducing cytoskeletal reorganization.

Figure 1: Schematic representation of Shigella invasion, intracellular replication and spreading in epithelial cells. Filopodial capture initiates the invasion process, accompanied with localized membrane ruffles at the epithelial cell apical surface. Following invasion, bacterial escape from the vacuole triggers, replicates freely in the cell cytosol and spreads from cell to cell using actin-based motility.

The Biology team at Collège de France led by Guy Tran Van Nhieu focuses on deciphering mechanisms in Shigella invasion of epithelial cells. In more recent developments, the work has been putting emphasis on the role of
mechanical forces and constraints during bacterial interaction with host cells that regulate of the invasion process [3].

Following internalization by epithelial cells, Shigella lyases the phagocytic vacuole to replicate freely in the cytosol, as illustrated in Fig. 1. Recent works have indicated that vacuolar lysis requires the recruitment of membrane compartments (Rab11-positive vesicles, macropinosome) as well as proteins such as the IpgD effector [4]. The mechanism leading to vacuolar rupture, however, remains ill defined. Because of the role of Ca\textsuperscript{2+} homeostasis in membrane repair, its implication during Shigella-induced vacuolar rupture was studied. By combining live imaging of vacuolar rupture and Ca\textsuperscript{2+} responses, the group at Collège de France has observed that bacterial invasion was associated with local Ca\textsuperscript{2+} increases (Fig. 2) [5,6]. These results suggested that vacuolar rupture required the local recruitment and/or activation of Ca\textsuperscript{2+}-dependent proteins during Shigella entry. Consistently, the Collège de France team has gathered evidence that myosin motors were recruited at the vacuolar membrane in a Ca\textsuperscript{2+}-dependent manner immediately before rupture. Furthermore, functional evidence points to a role for these myosins in vacuole upholding at the rupturing point and/or during escape. These results suggest that tethering of the vacuole to the cortical actin network is required for efficient rupture and depends on myosins, which would represent a paradigm shift for vacuolar lysis by intracellular pathogens (Fig. 2).

![Figure 2: Fluorescence micrographs of Shigella invasion foci on cultured intestinal epithelial cells. Blue: bacteria, Green: actin, red: talin. Bar is 10 μm.](image)

The PhD project will aim at characterizing the role of myosins in the establishment of constraints generated at the bacteria-containing vacuole, pertinent to the mechanisms of Shigella invasion and vacuolar escape. To address this issue, we will apply a novel microrheology technique that allows quantifying both the viscosity and the elasticity in complex fluids in general and in cells in particular.

**Cell Biomechanics: a probe microrheology approach**

Rheology is the study of flow and deformation of fluids on material sample of volume of the order of milliliters when they are submitted to mechanical stresses. Microrheology in contrast uses nano to micron size probes embedded in the material and needs much less sample, of the order of 1 picoliter. The past 20 years have seen increasingly rapid advances in this field, and
most noticeably in cell biomechanics [7]. Although such strategies provide reasonable insights on cell biomechanics, they face inaccurate descriptions of the cell heterogeneities and of the broad cell-to-cell variability.

To circumvent these limitations, the group at Laboratoire Matière et Systèmes Complexes led by Jean-François Berret (Université Paris-Diderot) designed novel probe particles in the form of elongated wires to perform passive and active microrheology in confined environments (Fig. 3) [8].

![Figure 3](image)

**Figure 3**: Electron and optical microscopy images of magnetic nanowires at different scales [8]. The wires are made from magnetic nanoparticle assembly. a) magnetic nanoparticles; b) a wire observed by TEM; c) wires observed by optical microscopy; d) wire dispersion.

Magnetic wires of diameter 0.5 - 1 µm and length 1 - 10 µm are produced following a bottom-up self-assembly method [8]. For microrheology, we exploit the rotational magnetic spectroscopy technique by placing a wire in a rotating magnetic field and monitor its temporal evolution by time-lapse microscopy. With this method, the static shear viscosity is obtained from the determination of a critical frequency between two rotation regimes, one where the wire and the field are synchronous, and one where the wire performs back-and-forth oscillations [9,10]. Recent cytoplasm viscosity measurements on murine NIH/3T3 fibroblasts, HeLa cervical cancer cells and A549 lung carcinoma epithelial cells were obtained with this method. It was first shown that the wires enter spontaneously into the cells without damaging them. Fig. 4 illustrates the entry mechanism of a 6 µm wire. Extensive microrheology assays were performed and confirmed the viscoelastic character of the cytoplasm, leading to cytosol viscosities of 10 – 100 Pa s and elastic moduli of 5 – 200 Pa [11]. Compared to other microrheology methods, the wire-based technique is simple to operate and needs minimal image treatment analysis.

**Objectives, originality and innovative nature of the project**

The PhD project will aim at characterizing the role of myosin in the establishment of constraints generated at the bacteria-containing vacuole, pertinent to the mechanism of *Shigella* vacuolar escape. The PhD work will involve physical chemistry, biophysics and biology experiments. More specifically, wire synthesis and rotational testing, preparation of in vitro infected cultured cells using a combination of molecular and cellular approach will be performed. In a second step, the measurements of viscosity parameters of the intracellular environment will be done to determine the effects of myosin on membrane tethering on *Shigella* invasion and spreading.
Figure 4: a) Internalization of a 6 µm magnetic wire in NIH/3T3 fibroblasts observed by optical microscopy. After its internalization, the wire is submitted to a magnetic torque that actuates its motion. From the torque value (between $10^{-18}$ and $10^{-16}$ N m) and the subsequent wire motion, the intracellular medium viscosity is determined. The static shear viscosity of fibroblasts could be estimated, providing a value of $47 \pm 20$ Pa s [11].

This will be performed through the following specific aims:

- The synthesis of magnetic/fluorescent nanowires.
- The measure of the deformability and viscosity on healthy epithelial cells to establish a reference behavior.
- The identification of the cytoskeleton network associated with cell mechanics.
- The determination of the role of myosins in the cell mechanical response using the wire rotation microrheology technique.
- The characterization of the role of myosins in the local cell cortex viscosity.
- The role of myosins in the constraints on the membranes of wires and bacteria-containing vacuoles.
- The identification of myosins ligands involved in mechanical constraints and membrane tethering to the actin cytoskeleton.

References


Résumé

*Shigella* is a bacterial pathogen responsible for bacillary dysentery in humans. *Shigella* has the ability to invade epithelial cells from the colon. The Biology team at Collège de France led by Guy Tran Van Nhieu focuses on deciphering mechanisms in *Shigella* invasion of epithelial cells. In recent work, this group has identified the role of mechanical forces and constraints during bacterial interaction with host, and in particular the role of myosins in the breaking of the bacteria enclosing vacuole. The PhD project will aim at characterizing the role of myosins in the establishment of constraints generated at the bacteria-containing vacuole, pertinent to the mechanism of *Shigella* vacuolar escape.

To tackle this issue, a novel microrheology technique allowing the measurement of both viscosity and elasticity of complex fluids will be applied. The group at Laboratoire Matière et Systèmes Complexes led by Jean-François Berret (Université Paris-Diderot) designed new probe particles in the form of elongated wires to perform passive and active microrheology in confined environments. This technique was successfully used in mammals cells recently and it confirmed the viscoelastic character of the cytoplasm.

The PhD work will involve physical chemistry, biophysics and biology experiments. More specifically, wire synthesis and rotational testing, preparation of in vitro infected cultured cells using a combination of molecular and cellular approach will be performed. In a second step, the measurements of viscosity parameters of the intracellular environment will be done to determine the effects of myosins in *Shigella* invasion and spreading.