aviesan

alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Conference on

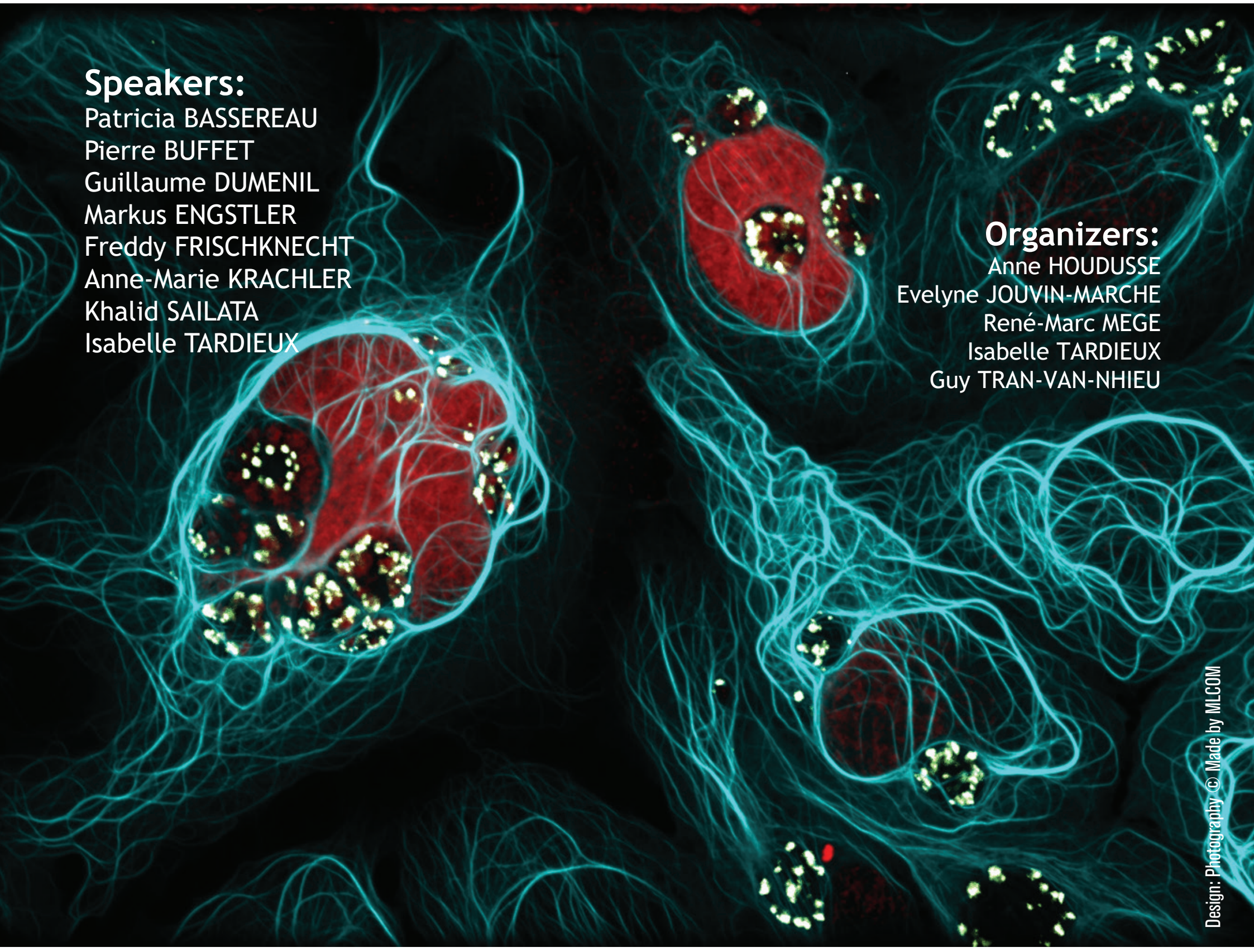
Mechanotransduction of Host-Pathogen Interactions

December 17th, 2018

Auditorium Biopark
11, rue Watt
Paris 13e, France

Speakers:
Patricia BASSEREAU
Pierre BUFFET
Guillaume DUMENIL
Markus ENGSTLER
Freddy FRISCHKNECHT
Anne-Marie KRACHLER
Khalid SAILATA
Isabelle TARDIEUX

Organizers:
Anne HOUDUSSE
Evelyne JOUVIN-MARCHE
René-Marc MEGE
Isabelle TARDIEUX
Guy TRAN-VAN-NHIEU



Free but compulsory registration on:
https://itbcde.aviesan.fr/

alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGY, INFLAMMATION, INFECTIOLOGY AND MICROBIOLOGY

ITMO CELL BIOLOGY, DEVELOPMENT AND EVOLUTION

Mechanotransduction of Host-Pathogen Interactions

December 17th, 2018

Auditorium Biopark
11, rue Watt
Paris 13e, France

9 :00 a.m. – 9 :30 a.m.	Welcome
9 :30 a.m. – 9 :40 a.m.	**Introduction by organizers**
	Session 1: Biophysics of Parasite-host cell interactions
9 :40 a.m. – 10 :15 a.m.	**Markus ENGSTLER**, University of Würburg, Germany "From solitary swimmers to swarms and back: trypanosomes on their journey through the tsetse fly and beyond"
10 :15 a.m. – 10 :30 a.m.	**Elisabeth LABRUYERE** et al, Pasteur Institute, Paris, France "A mechano-imaging method to quantify intracellular biophysics"
10 :30 a.m. – 11 :05 a.m.	**Pierre BUFFET**, Integrated red cell biology, Paris, France "Malaria, red cells and red spleen: from mechanical friction to controllable infection?"
11 :05 a.m. – 11 :25 p.m.	Coffee Break
11 :25 a.m. – 12 :00 p.m.	**Isabelle TARDIEUX,** Institute for Advanced Biosciences, Grenoble, France "The Toxoplasma tour de force to unfold the intravacuolar developmental program in metazoan cells"
12 :00 p.m. – 12 :15 p.m.	**Julien ROBERT-PAGANIN** et al, Curie Institute, Paris, France "The atypical and tunable force generation mechanism of *Plasmodium* class XIV myosin drives parasite invasion"
12 :15 p.m. – 12 :50 p.m.	**Friedrich FRISCHKNECHT,** Parasitology Heidelberg University, Heidelberg, Germany "Forces and shape changes of *Plasmodium* sporozoites during transmission of malaria"
12 :50 p.m. – 1:05 p.m.	**Eloïse BERTIAUX** et al, Pasteur Institute, Paris, France

	"Bidirectional intraflagellar transport is restricted to two sets of microtubule doublets in the trypanosome flagellum"
1 :05 p.m. – 2 :15 p.m.	Lunch break with posters
	Session 2: Biophysics of bacterial-host cell interactions
2 :15 p.m. – 2 :50 p.m.	**Anne-Marie KRACHLER**, McGovern Medical School, Texas, USA "Biophysics of bacterial adhesion and virulence"
2 :50 p.m. – 3 :05 p.m.	**Claude LOVERDO** et al, Laboratoire Jean Perrin, Paris, France "Antibody-mediated enchainment of bacteria in the gut : a possible mechanism for microbiota homeostasis"
3 :05 p.m. – 3 :40 p.m.	**Khalid SALAITA,** Emory University, Atlanta, Georgia, USA "Biophysics, nanoscience, force sensors at the cell membranes"
3 :40 p.m. – 3 :55 p.m.	**Nathalie SAUVONNET** et al, Pasteur Institute, Paris, France "Mechanical forces and 3D topology of the colonic epithelium are critical for Shigella infection using a biomimetic human gut on a chip"
3 :55 a.m. – 4 :15 p.m.	Coffee Break
4 :15 p.m. – 4 :50 p.m.	**Patricia BASSEREAU,** Curie Institute, Paris, France "Force measurements and giant unil amellar vesicles to study pathogen-host cell interactions"
4 :50 p.m. – 5 :05 p.m.	**Alexandra ZAK** et al, Ecole Polytechnique, Palaiseau, France "Viscoelastic properties of neutrophil during phagocytosis"
5 :05 p.m. – 5 :40 p.m.	**Guillaume DUMENIL,** Pasteur Institute, Paris, France "Neisseria meningitidis vascular colonization"
5 :40 p.m. – 5 :45 p.m.	Conclusion

Patricia Bassereau, Curie Institute, France

Patricia Bassereau is CNRS Directrice de Recherche (equivalent to professor) at the Institut Curie in Paris where she is the leader of the group "Membranes and cellular functions". She obtained a short PhD and a PhD in Soft Matter at the University of Montpellier where she started her carrier on the structure of self-assembled surfactant-based systems. She spent one year as a visiting scientist at the IBM Almaden Center (San Jose, USA) on thin polymer films. She moved to the Institut Curie in 1993 to work on questions related to "Physics of the cell". She develops a multidisciplinary approach, largely based on synthetic biology and biomimetic systems, as well as quantitative mechanical and microscopy methods to understand the role of biological membranes in cellular functions. Additionnally, she studies in vitro and in cellulo the mechanics and the generation of cellular protrusions.

Mechanics measurements and Giant Unilamellar Vesicles to study pathogen-host cell interactions

Many bacteria interfere with the actin cytoskeleton of the host cells for triggering invasion. This is the case of Shigella that interacts with very dynamical thin tubular cellular protrusions called filopodia or Staphylococcus aureus that induces trans-cellular tunnels ("transendothelial cell macroapertures" (TEM)). These pathogen-host cell interactions reveal new properties of actin assemblies in cells. In particular that filopodia and TEMs have common properties: a) filopodia are sustained by actin bundles, while the opening of TEM is limited by a stiff actin cable at its periphery; b) both contain I-BAR domains proteins and ezrin connecting plasma membrane to the actin filaments, which are essential for these structures. Here, we will address the question of the origin of the localization of these proteins on curved membranes. With in vitro experiments using Giant Unilamellar Vesicles (GUVs), purified proteins and membrane nanotubes of controlled curvature pulled from these GUVs, we have evidenced that the I-BAR domain protein IRSp53 is enriched on negatively curved membrane (Prevost et al., 2015), in agreement with the in vivo observations (Mattila and Lappalainen, 2008), but ezrin is not (Tsai et al., in preparation). However, we show that ezrin can be enriched on negatively curved membrane through its direct interaction with the I-BAR domain (Tsai et al., in preparation). Our work provides a mechanism to target ezrin to specific curved area of the plasma membrane, irrespectively of its large abundance at the plasma membrane.

Pierre Buffet, Integrated red cell biology, Paris, France.

Pierre Buffet is professor of Cell Biology at Paris Descartes University & Institut National de la Transfusion Sanguine, and consultant physician at Institut Pasteur. He has coordinated clinical trials and surveys in the treatment of leishmaniasis and malaria. After a PhD in microbial biochemistry at Institut Pasteur (Scherf Lab), he has explored the interaction between red blood cells and the spleen. He was head of the Medical Center and Clinical Research Center of Institut Pasteur from 2001 to 2007 then Associate Professor of Parasitology-Mycology at Salpêtrière Hospital from 2007 to 2015. He became head of an INSERM – Paris University research team in 2014. He has been coordinating more than 10 national and international research projects and supervised 30 students including Master, PhD and Post-doctoral students as well as residents and fellows. He was awarded the Research Excellence award of Paris 6 University in 2012 and the Infectious Diseases award from the Fondation pour la Recherche Médicale in 2018.

Malaria red cells and red spleen : from mechanical friction to controllable infection?

With colleagues at Institut Pasteur, we designed and validated a filtration method that mimics the sensing of stiff red blood cells by the spleen. This biomimetic device increases the throughput for quantification of red blood cells deformability by more than one order of magnitude. Screening for new classes of antimalarial drugs that will induce the mechanical retention of parasitized red blood cells in the spleen is ongoing and the first hits have just been identified. This novel approach (« parasite retention rather than parasite killing ») will predominantly target malaria transmission. The original idea stems from earlier results obtained in the human spleen perfusion model where we had observed the unexpected, purely mechanical retention of a proportion of parasitized red blood cells. This very original mechanism of innate control of a microbial proliferation – different from more conventional cell-microbe interactions involving cells of the innate or adaptive immune system – should help interpret genetic association studies in malaria in particular and exploration of malaria pathogenesis in general. We recently observed interethnic differences in red blood cell deformability related to infection with malaria parasites in Africa. The general approach thus connects red blood cell and spleen physiology to malaria syndromes and will hopefully contribute to malaria elimination.

Guillaunme DUMENIL, Institut Pasteur, Paris

Guillaume Dumenil did his PhD in P. Sansonetti team at the Pasteur Institute. Then, he went at Tufts Medical school for a post doct in Ralph Isberg's group for 3 years. Back in Paris, he joined the group of Xavier Nassif at the Necker hospital and since 2009, he is a group leader, initially, at the Paris Cardiovascular research center (PARCC) and since 2015 , head of the unit of Pathogenesis of Vascular Infections at the Pasteur Institute. He was awwarded the « Grand Prix de médecine et de recherche médicale "Jean Hamburger" de la Ville de Paris » and « the Médaille Louis Pasteur de l'Académie des Sciences »

More information at https://research.pasteur.fr/fr/member/guillaume-dumenil/

Neisseria meningitidis vascular colonization

Bacterial infection of human vasculature can lead to unregulated systemic activation of coagulation and innate immunity and rapidly becomes life threatening. *Neisseria meningitidis* is a vascular pathogen that causes fatal septic shock and meningitis. Post-mortem histological analysis of tissues from individuals infected with *N. meningitidis* show large bacterial aggregates in close association with the vascular wall of small vessels. The ability of this bacterium to colonize blood vessel endothelium is likely to impact its capacity to both multiply in the blood stream and reach the brain. This process is referred to as *vascular colonization*. Recent work from our group exploring the early steps in *N. meningitidis* vascular colonization will be reported, from attachment to proliferation and dissemination, focusing on the the physical dimension of bacterial–host interaction.

Markus Engstler, University of Würzburg, Germany

Markus Engstler is a molecular cell biologist with substantial interest in infection biology and (bio)physics. He graduated from Christian-Albrechts-University of Kiel where he also received a doctorate in biochemistry. Markus spent his postdoctoral time at The Rockefeller University (New York) and the Max Planck Institute of Biochemistry (Martinsried). He further developed his quantitative approaches to study cells as a project group leader at the Free University of Berlin and the Ludwig-Maximilians-University of Munich. Following habilitation in Genetics (LMU Munich), he was appointed Professor of Genetics at Darmstadt University of Technology. Since 2009, Markus Engstler is Professor and Chair of the Department of Cell and Developmental Biology at the Julius-Maximilians-University of Würzburg. Among other duties, he continues to act as a member of numerous scientific advisory boards, as well as an editor for several journals. He has served as vice-dean and dean of faculty, and is founding director of the Center for Computational and Theoretical Biology (CCTB). Markus Engstler is also a founding member of DNTDs, the German Network Against Neglected Tropical Diseases, and Vice-chairman of the German Society for Parasitology.

From solitary swimmers to swarms and back: trypanosomes on their journey through the tsetse fly and beyond

Parasitic life is characterized by close physical contact between pathogen and host. This particularly true for single-celled parasites that thrive in the mammalian circulation, such as African trypanosomes. Trypanosomes are flagellate microswimmers and causative agents of deadly human and animal diseases in the poorest parts of Africa. The parasites swim freely in the blood and tissue fluids of their mammalian hosts, where they employ hydrodynamic drag to escape immune destruction. Cell motility is essential for trypanosome survival, not only in the mammal, but also in the transmitting insect, the blood sucking tsetse fly.

My lecture discusses how these parasites may have adapted to the varying physical conditions in their hosts. Three basic aspects will be considered, namely membrane protein diffusion, cell motility and mechanotransduction. Trypanosomes exploit these processes for survival in the host, and their strategies may shed new light on some fundamental aspects of biophysics.

Friedrich FRISCHKNECHT, Heidelberg, Germany

Freddy studied Biochemistry at the Free University of Berlin and worked on the cell-to-cell spread of vaccinia virus for his PhD at EMBL Heidelberg. He first worked on Plasmodium during his postdoc at the Institut Pasteur, where he imaged the transmission of malaria parasites together with Rogerio Amino. In 2005 he started his own lab at the University of Heidelberg where he investigates the motile forms of the malaria parasite using state of the art light and electron microscopy, molecular genetic and biophysical tools. His lab is part of the collaborative research center "Integrative analysis of pathogen replication and spread" (www.sfb1129.de). More information about his work on www.sporozoite.org.

Forces and shape changes of Plasmodium sporozoites during transmission of malaria

Plasmodium parasites are the causative agents of malaria. They are transmitted to and by mosquitoes and need to be motile to enter and cross different types of tissues. To do so they evolved a gliding motility machinery that allows them to migrate at speeds of microns per second, an order of magnitude faster than mammalian cells. The talk will elaborate strategies to understand this motility by genetic manipulation, microscopy and force measurements.

Recent publications featuring in the talk:

Douglas et al., 2018. Inter-subunit interactions drive divergent dynamics in mammalian and *Plasmodium* actin filaments, *PLoS Biology*, 16:e2005345.

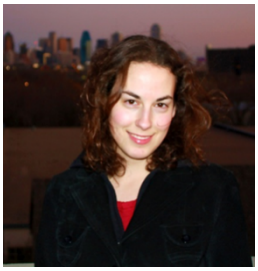
Klug and Frischknecht, 2017. Motility precedes egress of malaria parasites from oocysts. *eLife* 6:e19157.

Moreau et al., 2017. A unique profilin-actin interface is important for malaria parasite motility, *PLoS Pathogens* 13:e1006412.

Bane et al., 2016. The actin filament-binding protein coronin regulates motility in Plasmodium sporozoites. *PLoS Pathogens* 12:e1005710.

Quadt et al., 2016. Coupling of retrograde flow to force production during malaria parasite migration. *ACS Nano* 10:2091-102.

Anne-Marie Krachler, McGovern Medical School, USA



Dr. Krachler joined the faculty of the McGovern Medical School, Department of Microbiology and Molecular Genetics at the University of Texas Health Science Center at Houston as an Associate Professor in 2016. She completed her undergraduate and masters degree in Chemical Engineering at the Vienna University of Technology, Austria, before training for a Ph.D. in Biochemistry and Biophysics at the University of York in the UK. After joining Kim Orth's lab at UT Southwestern Medical Center as a postdoctoral fellow, she moved to the University of Birmingham as an EMBO Fellow and Birmingham Fellow in 2012, where she first established her independent laboratory.

Dr Krachler's research on bacteria-host interactions has been recognized by several awards, including a Texas Rising STAR award (2016), the Biochemical Society's Early Career Research Award for Biological Systems (2015) and a Founder's Award (2015). Her laboratory is currently funded by the Biotechnology and Biological Sciences Research Council and the National Institutes of Health.

May the force be with you:

Mechanoregulation of virulence genes in enterohemorrhagic *E. coli* O157

Foodborne infection with the human pathogen enterohemorrhagic Escherichia coli (EHEC) can result in outbreaks of bloody diarrhea and, in severe cases, hemolytic uremic syndrome. Cattle are the main reservoir of EHEC, and infection occurs following exposure to contaminated meat, dairy, or vegetables. Recently, we found that virulence genes in EHEC are not only regulated by chemical cues, but also mechanical cues derived from the environment. We have established a vertebrate model for foodborne EHEC infection using larval zebrafish (Danio rerio) as a host and the protozoan prey Paramecium caudatum as a vehicle. This model allows us to study intestinal colonization, microbial gene induction, and its dependence on mechanical forces within a live host. I will discuss bacterial factors required for mechanoregulation, their mode of action during mechanical induction, and the bacterial mechanoregulon specific to virulence and beyond.

Khalid Salaita, Emory University, USA

Khalid obtained his Ph.D. with Prof. Chad Mirkin at Northwestern University in 2006. During that time, he studied the electrochemical properties of organic adsorbates patterned onto gold films and developed massively parallel scanning probe lithography approaches. From 2006-2009, Khalid was a postdoctoral scholar with Prof. Jay T. Groves at UC Berkeley. As a postdoc, he developed electrostatic-based approaches for DNA microarray readout, and also investigated the role of EphA2 (RTK) receptor clustering in modulating cell signaling. In 2009, Khalid started his own lab at Emory University, where he integrates materials science and nanotechnology with biology. His group pioneered the development of molecular force probes and nano-mechanical actuators that are integrated with living cells. His group also develops DNA-based machines for chemical sensing as well as catalytic nanoparticles for gene regulation. In recognition of his independent work, Khalid has received a number of awards, most notably: the Alfred P. Sloan Research Fellowship, the Camille-Dreyfus Teacher Scholar award, and the NSF Early CAREER award, and has been selected as a Kavli Fellow. Khalid's program is supported by NSF, NIH, and DARPA.

A measure of molecular muscle: molecular probes to control and map forces in living cells

A major challenge to understanding the role of forces in cell biology pertains to the lack of molecular tools that allow one to image and manipulate forces at membrane receptors. To address this issue, we have developed a set of fluorescent probes (mechanophores) and actuators to investigate the role of forces in biochemical signaling. In this talk, I will highlight our work developing molecular force probes and their application in the area of mechanotransduction. Force probes take advantage of FRET to determine the extension of a molecular "spring" to quantify tension. Tension probes are modular and the spring element can be engineered using PEG polymers, oligonucleotides, and proteins. The latest generation of tension probes employ FRET, FLIM, and fluorescence anisotropy methods, which provide significant improvement in sensitivity. I will also describe the application of these probes in the study of integrin receptor mechanotransduction and T cell receptor activation. I will also demonstrate mechano-pharmacology applications of these nanoparticle force probes. Super-resolution imaging provide the highest resolution maps of cell mechanics. In the context of T cell mechanotransduction, I will describe the physical basis of T cell receptor recognition. Finally, I will discuss the development and application of optically controlled nanoscale actuators to control receptor tension, thus demonstrating an example of using light to physically control cell migration.

Isabelle Tardieux, Institute For Advanced Biosciences, Inserm U1209 Cnrs Umr 5309, Univ. Grenoble Alpes, France.

Isabelle Tardieux is a CNRS 1st class research director who currently heads a team working on « Cell Membrane Dynamics of Host-Parasite Interactions" in the Department of Prevention and Therapy of Chronic Diseases at the Institute for Advanced Biosciences (IAB) at Grenoble (France). She got a PhD in Population Dynamics and Entomology before being appointed as assistant professor at the Pasteur Institute (Paris, France) working on vector-borne diseases. She has been successively trained in Cell biology and Parasitology at Yale University (Infectious Disease Department, USA) and at the NIH Bethesda (Parasitic Diseases laboratory, USA) as post-doctoral fellow and visitor associate, respectively. She got extensive experience on membrane and cortical actin dynamics and in live high resolution imaging of parasite-cell interactions. Her team has recently moved towards cell biomechanics in tight collaboration with the biophysicists from the IAB and the laboratory of interdisciplinary Physics at Grenoble.

The *Toxoplasma* Tour de Force to unfold its intra-vacuolar developmental program in metazoan cells

The several micron-sized Toxoplasma gondii microbe is an obligate intracellular single-celled parasite that injects a multi-unit nanodevice into the target cell Plasma Membrane (PM). This "invasive" nanodevice promotes the assembly of a toroidal junction that transiently ties the two cells and directs the folding of an unconventional Entry Vesicle (EV) surrounding the invading parasite. Real time tracking of a core component of the parasite device, genetically fused to a fluorescent tag, has allowed us demonstrating that Toxoplasma applies an actin-myosin traction force on this junction while forcing budding of the EM. Recent spinnink disk confocal microscopy allowed capturing the stretched behavior of the torus immediately post-insertion in the host cell PM and throughout entry. Image processing unveiled a parasite-driven torque, which applies asymmetric forces on the nanodevice and mechanically promotes membrane scission upstream to it to ensure release of the parasite within a protective Parasitophorous Vacuole (PV). While proper torus closure is required for early remodeling of the PV membrane to further adjust to Toxoplasma growth requirements, interfering with this step leads to osmotic damages and parasite death. Since the fission proceeds independently of the host cell Dynamin or ESCRT-III families, we propose that Toxoplasma has evolved a toroidal nanodevice that responds to torsional and contractile forces thereby mimicking the contractile helices made by Dynamins or ESCRT-III oligomers to drive membrane fission in metazoans.

Organisateurs administratifs	Imane	**LAHMAMI**		Inserm	Paris	France	Imane.lahmami@inserm.fr
	Christine	**LEMAITRE**		Inserm	Paris	France	Christine.lemaitre@inserm.fr
	Sylvie	**ROBINE**	Dr	Inserm	Paris	France	sylvie.robine@inserm.fr
Organisateurs scientifiques	Thierry	**GALLI**	Dr	IPNP - ITMO BCDE	Paris	France	thierry.galli@inserm.fr
	Anne	**HOUDUSSE**	Dr	Institut Curie	Paris	France	anne.houdusse@curie.fr
	Evelyne	**JOUVIN-MARCHE**		Institute for Advanced Biosciences -ITMO I3M	Grenoble Paris	France	evelyne.jouvin-marche@inserm.fr
	René-Marc	**MEGE**	Dr	Institut Jacques Monod	Paris	Francre	rene-marc.mege@ijm.fr
	Isabelle	**TARDIEUX**		Institute for Advanced Biosciences	Grenoble	France	isabelle.tardieux@gmail.com
	Guy	**TRAN VAN NHIEU**	PhD	College de France	Paris	France	guy.tran-van-nhieu@college-de-france.fr
Selection short Talks	Eloise	**BERTIAUX**	PhD	Insritut Pasteur	Paris	France	eloise.bertiaux@pasteur.fr
	Elisabeth	**LABRUYERE**	PhD	Institut Pasteur	Paris	france	elisabeth.labruyere@pasteur.fr
	Claude	**LOVERDO**	Dr.	Sorbonne Université / CNRS	Paris	France	claude.loverdo@upmc.fr
	Julien	**ROBERT-PAGANIN**	Dr	Institut Curie	Paris	France	julien.robertpaganin@gmail.com
	Nathalie	**SAUVONNET**	Dr	Institut Pasteur	Paris	France	nathalie.sauvonnet@pasteur.fr
	Alexandra	**ZAK**	Madame	Ecole polytechnique-CNRS	Palaiseau	FRANCE	alexandra.zak@ladhyx.polytechnique.fr
ORATEURS	Patricia	**BASSEREAU**	Dr	Institut Curie	Paris	France	patricia.bassereau@curie.fr
	Pierre	**BUFFET**	Pr	Université Paris Descartes	Paris	France	pabuffet@gmail.com
	Guillaume	**DUMENIL**	Dr	Institut Pasteur	Paris	France	guillaume.dumenil@pasteur.fr
	Markus	**ENGSTLER**	Pr	University	Würzburg	Allemagne	markus.engstler@biozentrum.uni-wuerzburg.de
	Friedrich	**FRISCHKNECHT**	Pr	Department of Infectious Diseases	Heidelberg	Allemagne	fratsch@gmail.com
	Anne-Marie	**KRACHLER**	Dr	McGovern Medical School	Houston	USA	Anne.Marie.Krachler@uth.tmc.edu
	Khalid	**SALAITA**	Pr	Emory University	Atlanta	USA	k.salaita@emory.edu
	Isabelle	**TARDIEUX**	Dr	Institute for Advanced Biosciences	Grenoble	France	isabelle.tardieux@inserm.fr
PARTICIPANTS	Daniel Isui	**AGUILAR SALVADOR**	PhD	Collège de France	Paris	France	daniel-isui.aguilar-salvador@college-de-France.fr
	Philippe	**BASTIN**	Dr	Institut Pasteur/Inserm	Paris	France	pbastin@pasteur.fr
	Eloise	**BERTIAUX**	PhD	Insritut Pasteur	Paris	France	eloise.bertiaux@pasteur.fr
	Viviane	**BALLOY**	Dr	Inserm	Paris	France	viviane.balloy@inserm.fr
	Jeanne	**BIGOT**	PhD student	Inserm	Paris	France	jeanne.bigot27@gmail.com
	Nicolas	**BORGHI**	Dr	CNRS-IJM	Paris	France	nicolas.borghi@ijm.fr
	Claude	**BOUCHEIX**	DR	Inserm	Villejuif	France	claude.boucheix@inserm.fr

Thanh	**BOULDOIRES**	Post-doc	Curie Institute	Paris	France	thanh-huong-juliette.ho-bouldoires@curie.fr
Sylvie	**BOUTTIER**	Associate Professor	Université Paris Sud	Châtenay-Malabry	France	sylvie.bouttier@u-psud.fr
Latifa	**BOUZHIR**	Ingenieur	INSERM UMR 1174	ORSAY	FRANCE	latifa.bouzhir@u-psud.fr
Florence	**BRODERS**	CR CNRS	Insttut Curie	Paris	France	florence.broders@curie.fr
Adrien	**CAMUS**	PhD	CirB	Paris	France	adrien.camus@college-de-france.fr
Alenka	**COPIC**	Dr	IJM, CNRS	Paris	France	alenka.copic@ijm.fr
Anne	**COUEDEL-COURTEILLE**	MCU	Université Paris 7-Institut COCHIN	Paris	France	anne.couedel@inserm.fr
David	**COURTIN**	Senior researcher	IRD	Paris	France	david.courtin@ird.fr
Juan Martín	**DAMBROSIO**			Paris	France	juan.d'ambrosio@ijm.fr
Antoine	**DAVYDOFF**	Mr	Sorbonne Université	PARIS	France	antoine.davydoff@gmail.com
Isabel	**DOS SANTOS SOUZA RIBEIRO GOMES**	PhD	Institut Cochin	Paris	France	isabel.gomes@inserm.fr
Jost	**ENNINGA**	Head of research unit	Institut Pasteur	Paris	France	jost.enninga@pasteur.fr
Benoit	**GAMAIN**	PhD	INSERM	Paris	France	benoit.gamain@inserm.fr
Tatiana	**GOROJANKINA**	Ingenieur	Neuro PSI	Orsay	France	gorojankina@inaf.cnrs-gif.fr
Philippe	**HUBER**	Dr	CEA	Grenoble	France	phuber@cea.fr
EDMOND	**KOPYA**	Mr	University of Yaoundé I	Yaoundé	Cameroon	edmondoev@yahoo.fr
Jessica	**KUI**	PhD Student	ESPCI	Paris	France	jessica.kui@espci.fr
Elisabeth	**LABRUYERE**	PhD	Institut Pasteur	Paris	france	elisabeth.labruyere@pasteur.fr
Mireille	**LAMBERT**	Dr	Inserm	Paris	France	mireille.lambert@inserm.fr
Gordon	**LANGSLEY**	Dr	INSERM	PARIS	FRANCE	gordon.langsley@inserm.fr
Catherine	**LAVAZEC**	Dr	CNRS	Paris	France	catherine.lavazec@inserm.fr
Marion	**LE BRIS**	IR	Institut Cochin	Paris	France	marion.le-bris@inserm.fr
Sigolene	**LECUYER**	Dr	CNRS-LIPhy	Grenoble	France	sigolene.lecuyer@univ-grenoble-alpes.fr
Claude	**LOVERDO**	Dr.	Sorbonne Université / CNRS	Paris	France	claude.loverdo@upmc.fr
souhila	**MEDJKANE**	Dr	Université Paris-Diderot	Paris	France	souhila.medjkane@gmail.com
Amel	**METTOUCHI**	Dr	Institut Pasteur - CNRS	Paris	France	amel.mettouchi@pasteur.fr
Geneviève	**MILON**	Dr	Institut Pasteur	Paris	France	genevieve.milon@pasteur.fr
Eric	**MOCOEUR**	Mr.	Institut Jacques Monod	Paris	France	e.mocoeur@laposte.net
Dihia	**MOUSSAOUI**	PhD	Institut Curie	Paris	France	Moussaoui.Dihia@gmail.com
Emmanuel	**MUKWELE**	Chef de service	Programme National d'éradication de la Dracunculose	Kinshasa	DR Congo	emukwele20@gmail.com

Anna	**MULARSKI**	Dr	Inserm	Paris	France	anna.mularski@inserm.fr
Xavier	**NASSIF**	Pr	Insitut necker enfants malades	Paris	France	xavier.nassif@gmail.com
Florence	**NIEDERGANG**	Dr	Inserm	Paris	France	florence.niedergang@inserm.fr
Georgios	**PAVLOU**	PhD Student	IAB Grenoble	Grenoble	France	pavlou.grgs@gmail.com
Serena	**PETRACCHINI**	Mrs	Institut Pasteur	Paris	France	serena.petracchini@pasteur.fr
Maria-Luisa	**PISANELLI**	Student	Institut Pasteur	Paris	France	maria-luisa.pisanelli@pasteur.fr
Nathalie	**PUJOL**	CNRS	CIML	Marseille	FRANCE	pujol@ciml.univ-mrs.fr
Clotilde	**RANDRIAMAMPITA**	Dr	Institut Cochin	Paris	France	clotilde.randriamampita@inserm.fr
Lorena	**REDONDO-MORATA**	Dr	Institut Biologie Lille Inserm	Lille	France	lorena.redondo@inserm.fr
Julien	**ROBERT-PAGANIN**	Doctor (PhD)	Institut Curie	Paris	France	julien.robertpaganin@gmail.com
Brice	**ROTUREAU**	Dr	Institut Pasteur	Paris	France	rotureau@pasteur.fr
Gédéon	**SAMBA**	Country Director	Guinéa Worm Eradication Program	Kinshasa	DR Congo	gedeonsamba@gmail.com
Nathalie	**SAUVONNET**	Dr	Institut Pasteur	Paris	France	nathalie.sauvonnet@pasteur.fr
Olivier	**SILVIE**	Dr	Inserm	Paris	France	olivier.silvie@inserm.fr
Yasmine	**SMAIL**	Master student	Institut Cochin	Paris	France	yasmine.smail@ens.fr
Lamine	**TALL**	Journaliste	Infos Lumiere	Paris	France	talllamine@yahoo.fr
Sébastien	**TREGUER**	Lead Data Science	La Paillasse	Paris	France	streguer@gmail.com
Marina	**VETTESE**	-	-	-	France	vmarina@hotmail.fr
Philippe	**VINCENDEAU**	Pr	University of Bordeaux	Bordeaux	FRANCE	philippe.vincendeau@u-bordeaux.fr
Laetitia	**VINCENSINI**	Dr	Sorbonne Uni	Paris	France	laetitia.vincensini@upmc.fr
Allon	**WEINER**	Dr.	Cimi-Paris	Paris	France	allon.weiner@sorbonne-universite.fr
Véronique	**WITKO**	Dr	Inserm U1016	Paris	France	veronique.witko@inserm.fr
Alexandra	**ZAK**	Madame	Ecole polytechnique-CNRS	Palaiseau	FRANCE	alexandra.zak@ladhyx.polytechnique.fr

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mecanotransduction of host-pathogens interactions

Poster abstracts

Daniel AGUILAR SALVADOR	Shigella flexneri IpaA promotes cell adhesion mediated by vinculin supra-activation
Eloïse BERTIAUX *	Bidirectional intraflagellar transport is restricted to two sets of microtubule doublets in the trypanosome flagellum
Jost ENNINGA	How to tear *Shigella* containing vacuoles apart – A link between organelles, Rabs and microtubules
Edmond KOPYA	Exploring the impact of glutathione S-transferase-based metabolic resistance on vector competence of *Anopheles funestus* for *Plasmodium falciparum*
Elisabeth LABRUYERE *	A mechano-imaging method to quantify intracellular biophysics
Gordon LANGSLEY	*Theileria* highjacks JNK2 into a complex with the macroschizont GPIanchored surface protein p104
Claude LOVERDO *	Antibody-mediated enchainment of bacteria in the gut : a possible mechanism for microbiota homeostasis
Amel METTOUCHI	The Rac ubiquitylation pathway tunes integrin mechanotransduction
Dihia MOUSSAOUI	Structural studies on the *Plasmodium falciparum* myosins: search for inhibitors to stop parasite invasion
Anna MULARSKI	Studies of primary human macrophage phagocytosis and adhesion using traction force microscopy
Julien ROBERT-PAGANIN *	The atypical and tunable force generation mechanism of *Plasmodium* class xiv myosin drives parasite invasion
Nathalie PUJOL	Coupling tension and innate immunity in *c. Elegans* Epidermis
Nathalie SAUVONNET *	Mechanical forces and 3D topology of the colonic epithelium are critical for *Shigella* infection using a biomimetic human gut on a chip.
Alexandra ZAK *	Viscoelastic properties of neutrophil during phagocytosis

* selection short talks

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

SHIGELLA FLEXNERI IPAA PROMOTES CELL ADHESION MEDIATED BY VINCULIN SUPRA-ACTIVATION

SURNAMES
AGUILAR SALVADOR, Daniel Isui ; VALENCIA GALLARDO Cesar ; TRAN VAN NHIEU Guy
Unité de Communications Intercellulaires et Infections Microbiennes.
CIRB. CNRS UMR 7241/ INSERM 1050.
Collège de France
11, Place Marcelin Berthelot
75005 Paris Cedex
FRANCE
e-mail: daniel.isui-aguilar.salvador@college-de-france.fr

Keywords: Shigella, mechanotransduction, vinculin, microfluidics, adhesion

Texte

The intestinal epithelium is an essential barrier that enteroinvasive bacteria need to breach to promote diseases. Shigella, the causative agent of bacillary dysentery, invades polarized intestinal cells by injecting a variety of pro-tein effectors through a Type III secretion system. Among these, the type III effector protein IpaA has been shown to be important for invasion. IpaA modifies the cytoskeleton organization at bacterial invasion sites to allow adhesion and facilitate the internalization of bacteria. IpaA contains three vinculin binding sites (VBSs), which act together to promote an unmatched activation of the focal adhesion protein vinculin, and its association with actin filaments. Recent evidence from our lab indicates that IpaA induces allosteric structural changes in vinculin that affect cell adhesion dynamics and strength, as quantified by imaging and microfluidics approach. The results obtained confirm that IpaA VBSs interact with vinculin in a novel fashion, leading to its supra-activation and promoting cell adhesion.

Bidirectional intraflagellar transport is restricted to two sets of microtubule doublets in the trypanosome flagellum

Eloïse Bertiaux[1,2*], Adeline Mallet[1,2,3*], Cécile Fort[1,2], Thierry Blisnick[1], Serge Bonnefoy[1], Jamin Jung[1], Moara Lemos[1], Sergio Marco[4,5], Sue Vaughan[6], Sylvain Trépout[4,5], Jean-Yves Tinevez[7,8] and Philippe Bastin[1]

Affiliations:

[1]Trypanosome Cell Biology Unit, INSERM U1201, Institut Pasteur, 25 Rue du Docteur Roux, 75015 Paris, France

[2]Université Pierre et Marie Curie Paris 6, Cellule Pasteur-UPMC, 25 rue du Docteur Roux, 75015 Paris, France

[3]UtechS Ultrastructural Bioimaging (Ultrapole), Institut Pasteur, 28 Rue du Docteur Roux, 75015 Paris, France

[4]Université Paris Sud, Université Paris-Saclay, CNRS UMR9187, F-91405 Orsay, France

[5]Institut Curie, PSL Research University, INSERM U1196, F-91405 Orsay, France

[6]Department of Biological and Medical Sciences, Faculty of Health and Life Science, Oxford Brookes University

[7]UtechS Photonic Bioimaging (Imagopole), Institut Pasteur, 28 Rue du Docteur Roux, 75015 Paris, France

[8]Image Analysis Hub, Institut Pasteur, 28 Rue du Docteur Roux, 75015 Paris, France

*These authors contributed equally

Summary

Intraflagellar transport (IFT) is the rapid bidirectional movement of large protein complexes driven by kinesin and dynein motors along microtubule doublets of cilia and flagella. Here we used a combination of high-resolution electron and light microscopy to investigate how and where these IFT trains move within the flagellum of the protist *Trypanosoma brucei*. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) analysis of trypanosomes showed that trains are found almost exclusively along two sets of doublets (3-4 and 7-8) and distribute in two categories according to their length. High-resolution live imaging of cells expressing mNeonGreen::IFT81 or GFP::IFT52 revealed for the first time IFT trafficking on two parallel lines within the flagellum. Anterograde and retrograde IFT occur on each of these lines. At the distal end, a large individual anterograde IFT train is converted in several smaller retrograde trains in the space of 3-4 seconds while remaining on the same side of the axoneme.

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

HOW TO TEAR *SHIGELLA* CONTAINING VACUOLES APART- A LINK BETWEEN ORGANELLES, RABs AND MICROTUBULES

LOPEZ MONTERO Noelia and ENNINGA Jost
Institut Pasteur, Dynamics of host-pathogen interactions, 25 rue du Dr Roux, 75724 Paris
jost.enninga@pasteur.fr
Keywords: Bacterial invasion, *Shigella*, vacuolar rupture, macropinosomes, force generation

Most intracellular bacteria hijack and modulate specific trafficking pathways of their eukaryotic hosts, which are regulated by small GTPases of the Rab family, to stabilize and transform their bacteria containing vacuoles (BCVs) into “safe hideouts”. Within their BCVs, bacteria are capable to avoid efficiently host defense responses and complete their life cycle. Interestingly, we have recently discovered that the cytosolic bacterium *Shigella flexneri* employs a similar strategy but to the opposite purpose: *Shigella* subverts vesicular trafficking in order to destabilize and disassemble its BCV and to escape into the host cytosol.

We employ a variety of cell biology tools, advanced light and electron microscopy techniques, *in vitro* reconstitution assays, and proteomic approaches to investigate the molecular mechanism of *Shigella*’s BCV rupture. We have found that *Shigella* subverts a subset of Rab GTPases involved in recycling pathways. In particular, Rab35 is recruited to BCV membranes, which makes it the first and only resident Rab GTPase identified so far at the *Shigella* BCV. Our preliminary results suggest that the atypical relocation of Rab35 at BCVs is dependent on bacterial induced post-translational modification. This initiates a molecular cascade that eventually leads to the generation of divergent forces on BCV membranes, which in turn provokes them to be stripped away from the bacterium. With this intricate strategy employed by *Shigella*, we provide new insights into unknown functions and dynamics of Rab GTPase proteins.

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Exploring the impact of glutathione S-transferase-based metabolic resistance on vector competence of *Anopheles funestus* for *Plasmodium falciparum*

Edmond **KOPYA** [1,5], Cyrille **NDO**[1,2,3,4*], Helen **IRVING**[2], Gareth **LYCETT**[2] and Charles S. **WONDJI**[2,4]

1. Institut de recherche de Yaoundé (IRY), Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 288, Yaoundé, Cameroun

2. Vector group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK

3. Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box 24157, Douala, Cameroon

4. Centre for Research in Infectious Disease (CRI), P.O. Box 13591, Yaoundé, Cameroon.

5. Faculty of Sciences, University of Yaoundé I, P.O. Box 337, Yaoundé, Cameroon.

* Corresponding author: cyrndo@yahoo.fr

Abtract

Background

Malaria vector control heavily relies on insecticide-based control interventions. However, the increased spread of insecticide resistance is a major threat to sustainable disease control. For the major malaria vector *An. funestus*, the extent to which such resistance notably metabolic resistance interferes with the development of the *Plasmodium* malaria parasite and their impact on overall malaria transmission remains uncharacterised. The present study was conducted in order to explore whether the L119F-GSTe2 mutation

conferring pyrethroid/DDT resistance could influence *P. falciparum* development in *An. funestus*.

Methods

F1 females progenies of *An. funestus* collected in Cameroon were experimentally infected using parafilm glass-feeding system and *P. falciparum* gametocytes collected from blood of children. Mosquitoes were dissected at day 7 post infection, and midguts were analyzed for detection and quantification of oocysts. The L119F-GSTe2 marker was genotyped after amplication of DNA extracted from carcasses of dissected mosquitoes, and infection parameters (prevalence and intensity of infection) were compared between different genotype. Besides, a fragment of the GSTe2 gene was sequenced and compared in infected and uninfected individuals.

Results

A total of 2168 mosquitoes from independent infection experiments were dissected at day 7 and genotyped. A non-uniform geographical distribution of L119F-GSTe2 resistance allele was observed with a higher frequence in Obout (80%) compared to Mibellon (30%). Analysis of infection parameters showed that prevalence of infection as significantly high in heterozygous (RS) and homozigous suscetible geneotype (SS) while infection intensity, represented by oocyst load in midguts, resulted in significant high values in homozygous resistant (RR) and heterozygous (RS) mosquitoes. Sequencing analysis didn't allow detecting any polymorphism associated with infection in field *An. funestus* populations.

Conclusions

Altogether, these results suggests that GSTe2-based metabolic resistance may affect the vectorial competence of resistant *An. funestus* mosquitoes to *P. falciparum* infection, but additional studies are needed before drawing further conclusion. Nevertheless, considering the importance of *An. funestus* in malaria transmission and the wide distribution of insecticide resistance in this mosquito, these results are of great concern for the epidemiology of malaria in sub-saharan Africa.

Keywords: *An. funestus*, GSTe2, *P. falciparum*

A MECHANO-IMAGING METHOD TO QUANTIFY INTRACELLULAR BIOPHYSICS

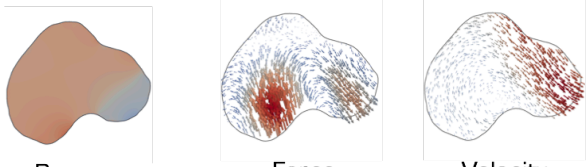
Elisabeth Labruyere [(1)], Aleix Boquet-Pujadas [(1)], Maria Manich [(1)], Nancy Guillen [(1)], Alexandre Dufour [(1)] and Jean-Chistophe Olivo-Marin [(1)]
[1]Bioimage Analysis Unit, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France
email: elisabeth.labruyere@pasteur.fr

Keywords: flow dynamics, forces, pressure, quantitative bioimaging, migrating cell.

Cellular movement in a tissue environment is generally triggered and/or guided by environmental stimuli (chemokine, nutrient or growth factor concentration gradient) and results in changes in cell morphology, which follow forces on the cell and within the cell.

Our project aims at developing an image based framework to model cell migration in complex 3D environments and to correlate cell morpho-dynamics features with intracellular events. To this end we have developed BioFlow, a method to extract intracellular biophysical quantities (flow dynamics, forces and pressure) using microscopy-imaging data (figure 1) [1].

Figure 1. Biophysics quantities measured with Bioflow at one time point of amoeboid migration (cell migration is rightwards)



We apply our method to analyse *Entamoeba histolytica* motility, a model for bleb-based amoeboid migration. Fluorescent amoebas were imaged with a spinning disk confocal microscope.

The spatiotemporal profile of the intracellular pressure during protrusion indicates that the pressure gradient precedes an increase in the cytoplasmic velocity. This supports the reported role of myosin II: an increase in pressure due to the contraction of the actomyosin cortex and, after the pressure stabilizes, a retraction of the cell rear accompanied by an increase in force. By studying the time between protrusions, we extend a period of 7.9s known only for confined *E. histolytica* to freely moving amoeba and discover a novel one of 4.6s that reflects the cytoplasmic streaming towards the bleb and could describe the actin cortex polymerization time at the edge of the protrusion. We showed that the pressure values differ across blebs, without disturbing the periodicity of bleb formation, indicating that pressure alone does not suffice to regulate bleb formation and stabilization. This is in agreement with recent evidence that bleb formation and regulation involves additional mechanisms such as Rho-GTPase activity. The importance of actin dynamics on intracellular flow, pressure and forces was showed by addition of Latranculin B leading to a stable decrease in the intracellular pressure gradient, velocity and force fields.

We expect to expand the reach of BioFlow by studying cell migration in 3D environments to describe the relationship between, shape and cytoskeleton dynamics and intracellular mechanics.

[1] *Boquet-Pujadas, A. et al. BioFlow: a non-invasive, image-based method to measure speed, pressure and forces inside living cells. Sci Rep, 2017.*

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

Theileria highjacks JNK2 into a complex with the macroschizont GPI-anchored surface protein p104

Gordon Langsley#, Perle Latré De Laté, Malak Haidar, Hifzur Ansari, Shahin Tajeri, Eszter Szarka, Anita Alexa, Kerry Woods, Attila Reményi, Arnab Pain.

#Laboratoire de Biologie Cellulaire Comparative des Apicomplexes, Faculté de Médecine, Université Paris Descartes - Sorbonne Paris Cité, France and Inserm U1016, Cnrs UMR8104, Cochin Institute, Paris, 75014 France.
Email: gordon.langsley@inserm.fr

Keywords: *Theileria annulata*; JNK2; JNK1; transformation; dissemination, autophagy

Constitutive JNK activity characterizes bovine T and B cells infected with *Theileria parva* and B cells and macrophages infected with *T. annulata*. Here, we show that *T. annulata* infection of macrophages manipulates JNK activation by recruiting JNK2 and not JNK1 to the parasite surface, whereas JNK1 is found predominantly in the host cell nucleus. At the parasite's surface JNK2 forms a complex with p104 a GPI-anchored *T. annulata* plasma membrane protein. Sequestration of JNK2 depended on PKA-mediated phosphorylation of a JNK-binding motif common to *T. parva* and a cell penetrating peptide harbouring the conserved p104 JNK-binding motif competitively ablated binding, whereupon liberated JNK2 became ubiquitinated and degraded. Cytosolic sequestration of JNK2 suppressed small mitochondrial ARF-mediated autophagy, whereas it sustained nuclear JNK1 levels, c-Jun phosphorylation and matrigel traversal. Therefore, *T. annulata* sequestration of JNK2 contributes to both survival and dissemination of *Theileria*-transformed macrophages.

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

Antibody-mediated enchainment of bacteria in the gut : a possible mechanism for microbiota homeostasis

Florence Bansept, Kathrin Schumann-Moor, Médéric Diard, Wolf-Dietrich Hardt, Emma Slack, Claude Loverdo*

* Laboratoire Jean Perrin, 4 place Jussieu, 75005 Paris, France

Keywords: Biophysics, Adaptive Immune system, Antibodies, Gut microbiota, Modelling

Immunoglobulin A is a class of antibodies produced by the adaptive immune system and secreted into the gut lumen to fight pathogenic bacteria. We recently demonstrated that the main physical effect of these antibodies is to enchain daughter bacteria, i.e. to cross-link bacteria into clusters as they divide, preventing them from interacting with epithelial cells, thus protecting the host. These links between bacteria may break over time. We study several models using analytical and numerical calculations. We obtain the resulting distribution of chain sizes, that we compare with experimental data. We study the rate of increase in the number of free bacteria as a function of the replication rate of bacteria. Our models show robustly that at higher replication rates, bacteria replicate before the link between daughter bacteria breaks, leading to growing cluster sizes. On the contrary at low growth rates two daughter bacteria have a high probability to break apart. Thus the gut could produce IgA against all the bacteria it has encountered, but the most affected bacteria would be the fast replicating ones, that are more likely to destabilize the microbiota. Linking the effect of the immune effectors (here the clustering) with a property directly relevant to the potential bacterial pathogeneicity (here the replication rate) could avoid to make complex decisions about which bacteria to produce effectors against.

THE RAC UBIQUITYLATION PATHWAY TUNES INTEGRIN MECHANOTRANSDUCTION

Amel Mettouchi[1, 2], Daniel Hamaoui[3], Anne Doye[2], Serena Petracchini[1], Atef Asnacios[4], Orane Visvikis[1,2], Patrick Munro[1,2], Elisa Vitiello[5], Martial Balland[5], Mukund Gupta[6], Benoit Ladoux[6, 7], Sebastien Janel[8], Franck Lafont[8], Jerôme Gilleron[9], Florian Fage[4], Petra Tafelmeyer[10], Laurent Gagnoux-Palacios[11], Mads Daugaard[12, 13], Poul H. Sorensen[14] and Emmanuel Lemichez[1, 2, 3]

1) Institut Pasteur, Bacterial Toxins Unit, 25-28 rue du Dr Roux 75724 Paris Cedex15.
2) INSERM, U1065, Université Côte d'Azur (UCA), Centre Méditerranéen de Médecine Moléculaire (C3M), Microbial toxins in host-pathogen interactions, Nice, France
3) Institut Pasteur, Cellular Biology of Microbial Infection
4) Laboratoire Matière et Systèmes Complexes, Unité Mixte de Recherche 7057, Centre National de la Recherche Scientifique and Université Paris–Diderot, Sorbonne Paris Cité, 75205 Paris, France
5) Univ. Grenoble Alpes, CNRS, LiPhy, F-38000 Grenoble, France
6) Mechanobiology Institute (MBI), National University of Singapore, Singapore 117411
7) Institut Jacques Monod (IJM), CNRS UMR 7592 & Université Paris Diderot, Paris, France
8) Cellular Microbiology and Physics of Infection Group, Center for Infection and Immunity of Lille, CNRS UMR8204, INSERM U1019, Institut Pasteur de Lille, Centre Hospitalier Régional de Lille, Université de Lille, Lille 59021, France
9)INSERM, U1065, UCA, C3M: Cellular and Molecular Physiopathology of Obesity and Diabetes, Nice, France
10) Hybrigenics Services, Paris, France.
11) Institut de Biologie Valrose (iBV), Université Côte d'Azur, CNRS UMR 7277, INSERM UMR 1091, Nice, France
12) Vancouver Prostate Centre, Vancouver, BC, V6H 3Z6, Canada.
13) Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada.
14) Department of Molecular Oncology, BC Cancer Research Center, University of British Columbia, Vancouver,BC V5Z1L3, Canada.

The small GTPase Rac1 is a critical cellular component hijacked by pathogenic enterobacteriaceae to promote cell and tissue invasion. We have established a novel host regulatory mechanism that restricts Rac1 activity through ubiquitylation and targeting to the proteasome once the GTPase is activated by the CNF1 toxin from pathogenic *E. coli*, by GEFs or point mutations. We identified the E3 ubiquitin ligase (E3L) HACE1 as the enzyme that catalyzes the ubiquitylation of active Rac1. HACE1 is a major tumor suppressor that limits Rac-dependent processes encompassing NADPH oxidase complex activity, S phase entry, cell migration, mammary cell transformation and tumor growth in animal models.

We identified Optineurin (OPTN) as a primary regulatory partner of the E3L HACE1. We report that OPTN is a new Extracellular matrix (ECM) stiffness sensor that activates HACE1 E3L activity. OPTN controls adhesion-mediated ubiquitin-proteasome degradation of Rac1 to tune Rac1 signaling with tissue stiffness. Loss of OPTN is associated with a gain of cell-ECM adhesive properties and enhanced integrin-mediated proliferative signaling and metabolic activity. Interestingly, cells that loose OPTN display atypical mechanical properties and apparent uncoupling of Focal Adhesions growth from actomyosin contractile activity. Together, our findings establish the first link between the Rac1 ubiquitylation pathway and ECM compliance sensing and define OPTN as a previously unknown mechanical sensor. We are now analyzing how bacteria can take advantage of CNF1 and cell tension forces to invade host cells.

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

Structural studies on the *Plasmodium falciparum* myosins: search for inhibitors to stop parasite invasion

MOUSSAOUI Dihia, Moussaoui.Dihia@gmail.com, Institut curie, 12 Rue L'homond, Paris.

Abstract:

Malaria is the deadliest parasitic disease (0.5 million deaths per year) and results from infection by Apicomplexan parasites from the genus *Plasmodium*. In this work, we show that PfMyoA, an atypical class XIV myosin from *Plasmodium falciparum,* is essential for the merozoite stage of the parasite to invade human erythrocytes. Crystallographic structures of PfMyoA coupled to transient kinetics experiments reveal the motor cycle of PfMyoA and to highlight the role of an atypical N-terminal extension that acts as a switch allowing the motor to move at high speed when the extension is phosphorylated (during motile stages) or to produce more force when dephosphorylated (during invasion). This study will pave the way for the design of a new generation of antimalarial drugs that could stall malarial invasion by blocking PfMyoA activity.

Keywords: Malaria, Myosin XIV.

Studies of primary human macrophage phagocytosis and adhesion using traction force microscopy

Anna Mularski, Artur Ruppel, Christophe Le Clainche, Martial Balland, Florence Niedergang

Phagocytosis is important for a variety of immune functions such as remodelling of tissues, disposal of dead cells and bacterial clearance. Actin polymerisation provides the force that drives the membrane deformation required to engulf particulate matter during phagocytosis. Key to dissecting the mechanism by which this occurs, is understanding how the complex mechanosensitive machinery of actin binding proteins sense force and stabilize actin anchoring during phagocytosis, while cells utilize this actin machinery to adhere to their environment. Traction force microscopy has been utilized to monitor force generation of phagocytosing and non-phagocytosing macrophages. This approach will be extended to study key actin binding proteins and help us to provide greater understanding of the role of these proteins in the related mechanosensitive processes of phagocytosis and adhesion.

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

THE ATYPICAL AND TUNABLE FORCE GENERATION MECHANISM OF *PLASMODIUM* CLASS XIV MYOSIN DRIVES PARASITE INVASION

Julien ROBERT-PAGANIN, James P. ROBLEE, Daniel AUGUIN, Thomas BLAKE, Carol S. BOOKWALTER, Elena B. KREMENTSOVA, Dihia MOUSSAOUI, Michael J. PREVIS, Guillaume JOUSSET, Jake BAUM, Kathleen M. TRYBUS, Anne HOUDUSSE

Structural Motility, UMR 144 CNRS/Curie Institute, 26 rue d'Ulm 75258 Paris cedex 05, France
julien.robert-paganin@curie.fr

Keywords: Malaria, Myosin A, glideosome, structural biology, drug design.

Malaria is the deadliest parasitic disease (0.5 million deaths per year) and results from infection by Apicomplexan parasites from the genus *Plasmodium*. In this work, we show that PfMyoA, an atypical class XIV myosin from *Plasmodium falciparum,* is essential for the merozoite stage of the parasite to invade human erythrocytes. Crystallographic structures of PfMyoA coupled to transient kinetics experiments reveal the motor cycle of PfMyoA and to highlight the role of an atypical N-terminal extension that acts as a switch allowing the motor to move at high speed when the extension is phosphorylated (during motile stages) or to produce more force when dephosphorylated (during invasion). This study will pave the way for the design of a new generation of antimalarial drugs that could stall malarial invasion by blocking PfMyoA activity.

aviesan
alliance nationale
pour les sciences de la vie et de la santé

ITMO IMMUNOLOGIE, INFLAMMATION, INFECTIOLOGIE ET MICROBIOLOGIE

ITMO BIOLOGIE CELLULAIRE, DÉVELOPPEMENT ET ÉVOLUTION

Mechanotransduction of Host-Pathogen Interactions

COUPLING TENSION AND INNATE IMMUNITY IN *C. ELEGANS* EPIDERMIS

PUJOL Nathalie[1], ESSMANN Clara[2], PUJOL Remy[3], CAZEVIELLE Chantal[3], EWBANK Jonathan[1]

1 Centre d'Immunologie de Marseille-Luminy, AMU, INSERM, CNRS, Marseille, France, pujol@ciml.univ-mrs.fr
2 UCL TouchLab, Department of Computer Science, University College London, Engineering Building, Malet Place, London, WC1E 7JG, UK
3 Institute for Neurosciences of Montpellier, Université Montpellier, INSERM, Montpellier, France.

Keywords: (5-6) Mechanosensation, injury, actin, AFM, electron microscopy

In nematodes, the cuticle forms an impermeable protective layer. It is intimately linked to the underlying syncytial epidermis and functions as an exoskeleton. It is a complex structure composed of different collagens and with circumferential annuli and furrows regularly spaced along the length of the animal. A new cuticle is formed at each moult in a process involving profound reorganisation of the cytoskeleton in the epidermis and sequential expression of collagen genes. A subset are expressed early and encode collagens found uniquely in furrows. Furrow-less mutants exhibit alterations of their cuticle and a constitutive activation of immune, osmotic, and detoxification responses. Physical injury of the cuticle also triggers an innate immune response in the epidermis. We hypothesise that these transcriptional stress responses are dependent upon a common sensor in the epidermis, potentially activated by mechanical stress and involving the furrow collagens (Dodd et al, 2018).

To investigate this possibility, we first used atomic force microscopy to acquire high-resolution images and simultaneously assess the mechanical properties of the cuticle. This revealed differences in cuticle furrow topography and in stiffness between wild-type worms and the cuticle mutants. By transmission electron microscopy, we observed that the mutant cuticle does not have its usual 3-layered structure, and was not tightly attached to the epidermis plasma membrane as in the wild type. Initial results also suggest that the mutants have an alteration in an organelle found specifically in the epidermis. These structures are composed of 4-10 parallel plasma membrane folds, forming saucer-like stacks, in intimate contact with the cuticle and often associated with a mitochondrion. They resemble eiosomes found in yeast. We are currently addressing the possibility that these organelles could play a role in damage sensing, coupling cuticle tension to signalling in the epidermis.

Mechanical forces and 3D topology of the colonic epithelium are critical for *Shigella* infection using a biomimetic human gut on a chip.

Alexandre Grassart[1], Anna Sartori-Rupp[2], Samy Gobba[3], Phillipe Sansonetti[1] and **Nathalie Sauvonnet**[1*].

[1] Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, INSERM U1202, [2] Unité Technologie et service Biolmagerie Ultrastructurale Institut Pasteur, [3] Biomaterial and microfluidics platform, Institut Pasteur
nathalie.sauvonnet@pasteur.fr

Key words
Organ on a chip, host-pathogen interactions, intestinal epithelium, microfluidics, peristalsis, shear forces, stretching.

Abstract

Intestinal epithelial cells are permanently exposed to mechanical forces and pathogens. Despite an increasing amount of knowledge on the molecular machineries elaborated by pathogens to manipulate the host cell, the role of mechanical forces on their interactions and invasion at the tissue level remains limited. This holds particularly true for S. flexneri, a human-restricted pathogen for which the initial steps of invasion is still unclear. Here, we employed an organ on chip device that mimic the colonic epithelial barrier with its physical forces to study S. flexneri invasion. Strikingly, we observed that only a few hundreds of bacteria were sufficient to infect enterocytes barrier when applied from the colon lumen. Moreover, by modulating independently the environmental cues, we found that the 3D architecture of the reconstituted tissue and the mechanical forces have a profound impact on bacterial invasion. In conclusion, our results reveal that Shigella takes advantage of the gut morphology and physical forces to disrupt the intestinal barrier.

VISCOELASTIC PROPERTIES OF NEUTROPHIL DURING PHAGOCYTOSIS

ZAK Alexandra[1,2],DUPRE-CROCHET Sophie [2],HUDIK Elodie[2],
BABATAHERI Avin [1],NUSSE Oliver[2],HUSSON Julien
[1]

[1]Laboratoire d'Hydrodynamique(LadHyX), CNRS UMR7646,
Ecole polytechnique, Palaiseau, France
[2]Laboratoire de Chimie Physique (LCP), CNRS UMR8000,
Université Paris Sud, Orsay, France
alexandra.zak@ladhyx.polytechnique.fr

Phagocytosis is one of the first steps of an immune response. To phagocyte, a neutrophil needs to surround the target with a phagocytic cup. While the cell is moving forward on the target, it organizes its cytoskeleton and exerts forces on its target. Although it has been shown that force generation correlates with an increase in cell tension, little is known about the viscoelastic cell properties related to these cytoskeletal rearrangements. Our goal is to measure both **elastic and viscous properties during the phagocytic cup progression around the target**.

We designed a **single-cell rheometer** to quantify changes in viscoelastic properties of non-adherent cells. This micropipette-based setup allows to combine morphological observations at single-cell level to mechanical measurements during phagocytosis both in the phagocytic cup and at the rear of the cell. To do so, we put in contact a **neutrophil with an antibody-coated 20-μm-diameter polystyrene bead** and apply a compressive force to the cell using a flexible cantilever. Once a desired level of force is reached, the cantilever oscillates and imposes oscillatory variations in force level (at a constant frequency of 1Hz) through an automated feedback. While the cell spreads on the bead, we are able to follow the evolution of the viscoelastic properties of the cell. We measure two mechanical properties of the neutrophil: the elastic part (K') and the viscous part (K") of its complex stiffness (both parameters are expressed in nN/μm).

Our results show that **during phagocytosis both stiffness and viscous properties increase in the phagocytic cup**. While the neutrophil spreads on an antibody-coated 20-μm diameter polystyrene bead, both stiffness and viscosity increase ten and four folds, respectively. These changes in viscoelastic properties during phagocytosis could explain the spreading dynamics and help understanding the role of cytoskeletal rearrangements in establishing a phagocytic cup.


Bibliothèque
François Mitterrand
RER C et M14
Université Paris Diderot
AUDITORIUM
11 rue Watt
BioPark
Aviesan-Inserm
8 rue Croix de Jarry
Hotel Park&Suites
bus 89, 62
Tram T3
Bibliothèque
universitaire des...
Rue du Chevaleret
Avenue de France
Rue Neuve Tolbiac
Rue René Goscinny
Quai Panhard et Levassor
Seine
Port de Bercy
Rue Cantagrel
Rue Léo Frankel
Rue Watt
Rue Albert Einstein
Rue du Loiret
Rue de Patay
Porte de
la Gare
Google