ONZIÈMES ENTRETIENS DE L'IBPC

Mercredi 13 avril - Vendredi 15 avril 2016

Mini symposium Labex Dynamo

Conférence Edmond de Rothschild

Journées organisées avec le soutien de :

ONZIÈMES ENTRETIENS DE L'IBPC

Les entretiens de l'IBPC ponctuent régulièrement la vie scientifique de l'institut. Ils représentent l'occasion pour l'ensemble des personnels statutaires et non statutaires de se retrouver pour découvrir la richesse et la diversité des recherches conduites au sein de l'IBPC. L'année 2016 verra la tenue des onzièmes entretiens de l'IBPC. Ceux-ci vont se dérouler sur trois jours, du mercredi 13 au vendredi 15 avril 2016, et seront couplés à un mini symposium du Labex Dynamo. Ces journées seront clôturées par les exposés des Lauréats 2016 du Prix Nine Choucroun et par la Conférence Edmond de Rothschild.

Cet événement important dans la vie de l'institut placé sous la direction de Francis-André Wollman, directeur de l'IBPC a été organisé par :

Comité d'organisation :

- Soumaya LAALAMI (UMR 8261)
- Christel LE BON (UMR 7099)
- Stefania VIOLA (UMR 7141)

Le comité scientifique composé de représentants statutaires et non statutaires de chaque unité :

- Gregory BOËL (UMR 8261)
- Yves CHOQUET (UMR 7141)
- Dario De VECCHIS (UPR 9080)
- Maude GUILLIER (UMR 8261)
- Julien HENRI (UMR 8226)
- Oana ILIOAIA (UMR 7099)
- Pierre-François PLUCHON (UMR 8226)
- Hager SOUABNI (UMR 7099)
- Guillaume STIRNEMANN (UPR 9080)
- Wojciech WIETRZYNSKI (UMR 7141)

Les membres de la FRC 550, tout particulièrement :

- Yasmine ABDELJALIL
- Yves DAPRA
- Catherine LARGET
- Franck PARASKIOVA

Un grand merci à Danielle BASCLE, Isabelle KREMPHOLTZ, Laurence GAUTHIER, Edith GODARD et Victoria TERZIYAN (gestionnaires des unités de l'IBPC), ainsi qu'aux doctorants et post-doctorants pour leur contribution à la réussite de cet évènement.

Mercredi 13 Avril 2016

9:00-9:20 Message de bienvenue par les membres du comité d'organisation et le directeur de l'IBPC

9:20-10:20 Présentation des Unités

FRC 550 : **Yasmine ABDELJALIL**
Laboratoire de Physiologie Membranaire et Moléculaire du Chloroplaste UMR 7141 : **Benjamin BAILLEUL**
Laboratoire de Biologie Physico-Chimique des Protéines Membranaires UMR 7099 : **Francesca ZITO**
Laboratoire de l'Expression Génétique Microbienne UMR 8261 : **Anaïs BROSSE**
Laboratoire de Biochimie Théorique UPR 9080 : **Guillaume STIRNEMANN**
Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes UMR 8226 : **Julien HENRI**

10:20-10:50 Pause café

Leslie DUTTON, Professeur invité Labex Dynamo

10:50-11:00 Modérateur : P. DERREMAUX
11:00-12:00 **Leslie DUTTON**
Toward the biogenesis of manmade light and redox proteins working in cells

12:00-13:50 Apéritif de bienvenue
Pause déjeuner libre

Organisation supramoléculaire des membranes transductrices d'Energie

Modérateur : Francis-André WOLLMAN

13:50-14:00 Présentation de la session
14:00-14:10 **Evocation de Fabrice RAPPAPORT par Francis-André WOLLMAN**
14:10-14:30 **Félix BUCHERT**
Biochemical analysis of the dynamic interaction between auxiliary proteins and core components of the CEF supercomplex
14:30-14:50 **Wojciech NAWROCKI**
Energy redistribution or dissipation? How do state transitions regulate energy transfer?
14:50-15:10 **Stefano SANTABARBARA** (Université de Milan, ITALIE)
Energy Transfer Between Light Harvesting Complex II and Photosystem I in the Supercomplex Formed During State Transitions.
15:10-15:30 **Adnan SAYEGH** (ENS, Paris, FRANCE)
Working out a photoanode using photosynthetic algae

15:30-16:00 Pause café

Structure et dynamique des protéines (1/2)

Modérateur : Christel LE BON

16:00-16:10 Présentation de la session
16:10-16:30 **Florian GEORGESCAULD**
Role of chaperones in mitochondria and chloroplasts protein import
16:30-16:50 **Marina KATAVA**
Computational investigation of the structure and the function of thermophilic proteins at high temperature
16:50-17:10 **Marina CASIRAGHI**
Functional modulation of a GPCR conformational landscape in a lipid bilayer
17:10-17:30 **Hager SOUABNI**
Use of functionalized amphipols for studying membrane protein supercomplexes by electron microscopy (EM)
17:30-17:50 **Pierre HARDOUIN** (Collège de France, Paris, FRANCE)
RNase Y, a new endoribonuclease involved in mRNA processing and degradation in *Bacillus subtilis*

Jeudi 14 Avril 2016

Structure et dynamique des protéines (2/2)

Modérateur : Philippe DERREUMAUX

9:00-9:10 Présentation de la session

9:10-9:30 **Martin PICARD**
Functional investigation of the MexA / MexB / OprM multidrug efflux pump from *Pseudomonas aeruginosa*

9:30-9:50 **Mara CHIRICOTTO**
Hydrodynamic effects on Amyloid-β aggregation

9:50-10:10 **Yoann LAURIN**
Structural variability and binding modes of the glioma targeting NFL-TBS.40-63 peptide on tubulin

10:10-10:30 **Charles BOU NADER** (Collège de France, Paris, FRANCE)
Recognition of tRNA by the human tRNA-Dihydrouridine synthase2: involvement of a double stranded RNA-binding domain

10:30-11:00 Pause café

Métabolisme et biologie des ARNs

Modérateur : Harald PUTZER

11:00-11:10 Présentation de la session

11:10-11:30 **Liuba MAZZANTI**
Integrative coarse–grained RNA modeling for small–angle X–ray scattering

11:30-11:50 **Albertas NAVICKAS**
Cytoplasmic fate of sense-antisense mRNA pairs

11:50-12:10 **Jonathan JAGODNIK**
Regulating bacterial gene expression with RNAs: the control of envelope composition and iron uptake

12:10-12:30 **Marina CAVAIUOLO**
The transcriptomic landscape of the Chlamydomonas chloroplast as revealed by sRNA-Seq and WTSS

12:30-14:00 Pause déjeuner libre

Traduction

Modérateur : Soumaya LAALAMI

14:00-14:10 Présentation de la session

14:10-14:30 **Caroline LACOUX**
RNA helicases: their structure, function and properties

14:30-14:50 **Gregory BOËL**
Functional characterization of a new class of protein translation factors belonging to the ABC-F protein family in *E. coli* and cyanobacteria

14:50-15:10 **Wojciech WIETRZYNSKI**
Characterization of Rubisco assembly-dependent regulation in *Chlamydomonas reinhardtii*

15:10-15:40 Pause café

Signalisation

Modérateur : Stéphane LEMAIRE

15:40-15:50 Présentation de la session

15:50-16:10 **Arnaud GAUTIER** (ENS, Paris, FRANCE).
A genetically encodable fluorescence on/off switch for advanced biomolecular imaging

16:10-16:30 **Marcello DE MIA**
Remodeling of the photosynthetic apparatus under Nitrogen and Sulfur starvation in *Chlamydomonas reinhardtii*

16:30-16:50 **Staëlle MAKAMTE**
The hedgehog signaling pathway: structural and functional studies of proteins Patched and SUFU

16:50-17:10 **Zhou XU**
Two routes to senescence in the absence of telomerase

Vendredi 15 Avril 2016

Régulation et prolifération membranaire
Modérateur : Bruno MIROUX

9:00-9:10	Présentation de la session
9:10-9:20	**Evocation de Philippe DEVAUX par Sophie CRIBIER**
9:20-9:40	**Dror WARSCHAWSKI** Watching lipids directly in the cell: fluorescence microscopy and solid-state NMR
9:40-10:00	**Federica ANGIUS** Molecular basis of membrane protein production and internal membranes proliferation in *Escherichia coli*
10:00-10:20	**Naima BELGAREH-TOUZÉ** Novel implications of the ubiquitin conjugation system in yeast mitochondrial dynamics and mitophagy
10:20-10:40	**Dario DE VECCHIS** Mitochondrial membrane fusion: computational modelling of mitofusins
10:40-11:10	**Pause café**

De la bactérie à l'organelle evolution
Modérateur : Stefania VIOLA

11:10-11:20	Présentation de la session
11:20-11:40	**Emmanuelle BOUVERET** (LISM, Marseille, FRANCE) Characterization of the ppGpp synthesis and degradation enzymes of *Chlamydomonas reinhardtii*
11:40-12:00	**Magali LEROY** Role of a new endoribonuclease YacP in selective mRNA turnover in *Bacillus subtilis*
12:00-12:20	**Anna LIPONSKA** RNase J of *Chlamydomonas reinhardtii*
12:20-14:20	**Pause déjeuner libre**

Lauréats Prix Nine Choucroun
Modérateur : Pierre JOLIOT

14:20-14:30	Présentation de la session
14:30-14:55	**Stanislas VON EUW**, Post-doctorant associé à l'Université de Rutgers (New Brunswick, NJ, USA). Bone biomineralization: from the structural characterization of the mineral to its 3D organization.
14:55-15:20	**Joël LEMIERE**, Post-doctorant Associé à l'Université de Yale (New-Haven, CT, USA). Actin cytoskeleton and membrane shaping: from liposome to cellular reconstitution.

Conférence Edmond de Rothschild
Modérateur : Francis-André WOLLMAN

15:20-15:30	Présentation de la session
15:30-17:00	Conférence Edmond de Rothschild

Professeur Mei HONG du département de Chimie du MIT (Cambridge, MA, USA)
Structure, Dynamics and Mechanism of Action of the Influenza M2 Protein from Solid-State NMR Spectroscopy.

17:00-20:00 **Cocktail**

Leslie DUTTON, Professeur invité Labex Dynamo

Toward the biogenesis of manmade light and redox proteins working in cells

P. Leslie Dutton

The Johnson Research Foundation, University of Pennsylvania, Philadelphia, USA

Bringing the idea of integrating manmade oxidoreductases and allied proteins into cell and organelle to replace or augment component parts of metabolic and bioenergetic machinery has some way to go to before any benefit to mankind can be expected. Nevertheless, once realized, the impact on solar energy conversion into chemical fuels, on remediating hemorrhagic traumas, post-operative reperfusion injuries, and on correcting respiratory dysfunction of genetic or aging origins will be considerable.

We combine first-principles photochemistry and electron transfer engineering with first-principles α-helical protein design, expression and cofactor assembly with a view to reproduce selected natural functions common to photosynthetic and respiratory energy conversion and oxidative metabolism. These simple, versatile manmade scaffolds, maquettes, prove to be controllably stable from 0 to >100C. Biocompatibility is demonstrated by co-expression and incorporation of cofactors, so far hemes B, covalently-linked heme C, bilins and indications of chlorophyll; also by ready specific transport across cellular membranes by Tat (transports folded proteins) or Sec (transports unfolded proteins).

With minor alterations, maquettes can be turned to promote diverse natural functions including a) light harvesting, energy transfer and charge-separation of photosynthesis, b) diffusive inter-protein electron-transfer to natural cytochrome *c* of photosynthesis and respiration, c) blue light-activated magnetic-field sensing recognized in cryptochromes thought to provide navigation in birds, and d) O_2 binding with properties of the globin O_2 transporters, now in animal trials. Co-expressed with selected bilins in mammalian cells these highly fluorescent in the near i.r maquettes show promise as optical probes. Finally, they lend themselves to nanoscale patterning on surfaces for light excitation energy transfer and electron transfer for clinical applications.

This work was supported by grant 'DYNAMO,' ANR-11-LABX-0011-01 from the French 'Initiative d'Excellence' program.

Organisation supramoléculaire des membranes transductrices d'Energie

- **Félix BUCHERT**
  Biochemical analysis of the dynamic interaction between auxiliary proteins and core components of the CEF supercomplex

- **Wojciech NAWROCKI**
  Energy redistribution or dissipation? How do state transitions regulate energy transfer?

- **Stefano SANTABARBARA**
  Energy Transfer Between Light Harvesting Complex II and Photosystem I in the Supercomplex Formed During State Transitions.

- **Adnan SAYEGH**
  Working out a photoanode using photosynthetic algae

Biochemical analysis of the dynamic interaction between auxiliary proteins and core components of the CEF supercomplex

Felix Buchert[1], Francis-André Wollman[1]*

[1] Institut de Biologie Physico-Chimique, UMR 7141 CNRS-UPMC, 13 rue P et M Curie, 75005 Paris, France
* wollman@ibpc.fr

Most of the energy in the kingdom of life relies on photosynthesis which allows the transient storage of solar energy as carbohydrates thanks to carbon dioxide fixation via the Calvin-Benson cycle[1]. To ensure the assimilation of fixed carbon, ATP and NADPH serve as energy currencies and are produced by photosynthetic electron transfer. Depending on environmental cues two indispensable modes exist, termed linear (LEF) and cyclic electron flow (CEF)[2]. While LEF provides ATP and NADPH, CEF balances out an increased demand for ATP and plays a photoprotective role. When respiration is inhibited in *Chlamydomonas reinhardtii* under anoxia, the intracellular reducing power increases, and thereby the plastoquinone pool is reduced. Thus, intracellular ATP/NADPH ratio is lowered and CEF is promoted. During CEF, electrons reenter the electron transport chain either at the plastoquinone pool or at the stromal side of the cytochrome b_6f complex. When switching between LEF and CEF, structural rearrangements of membrane proteins occur which were supported by isolation of a CEF supercomplex from *Chlamydomonas reinhardtii*[3]. The supercomplex consists of photosystem I, cytochrome b_6f, light harvesting complexes from both photosystems, and various auxiliary proteins of which the functional role still remains elusive. In the solubilized state the isolated supercomplex is rather instable and, so far, no structural information is available.

Here, we examine the effect of the detergent concentration during the rate-zonal centrifugation step using sucrose density gradients. We found that cytochrome b_6f complex was detected in lower density fractions if the detergent concentration of the sucrose gradient was increased. This indicated that high detergent concentration had a destabilizing effect on the CEF supercomplex which could be partially compensated by adding a crosslinker prior solubilization of the membrane proteins. Making use of the crosslinker, the auxiliary CEF supercomplex protein anaerobic response 1 (ANR1)[4] as well as PETO appeared to be crosslinked to cytochrome b_6f complex, suggesting close spatial localization in the CEF supercomplex.

References

[1] Arnon, D. I. (1959) Conversion of light into chemical energy in photosynthesis, Nature 184, 10-21.

[2] Foyer, C. H., Neukermans, J., Queval, G., Noctor, G., and Harbinson, J. (2012) Photosynthetic control of electron transport and the regulation of gene expression, Journal of experimental botany 63, 1637-1661.

[3] Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y., and Minagawa, J. (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis, Nature 464, 1210-1213.

[4] Terashima, M., Petroutsos, D., Hudig, M., Tolstygina, I., Trompelt, K., Gabelein, P., Fufezan, C., Kudla, J., Weinl, S., Finazzi, G., and Hippler, M. (2012) Calcium-dependent regulation of cyclic photosynthetic electron transfer by a CAS, ANR1, and PGRL1 complex, Proceedings of the National Academy of Sciences of the United States of America 109, 17717-17722.

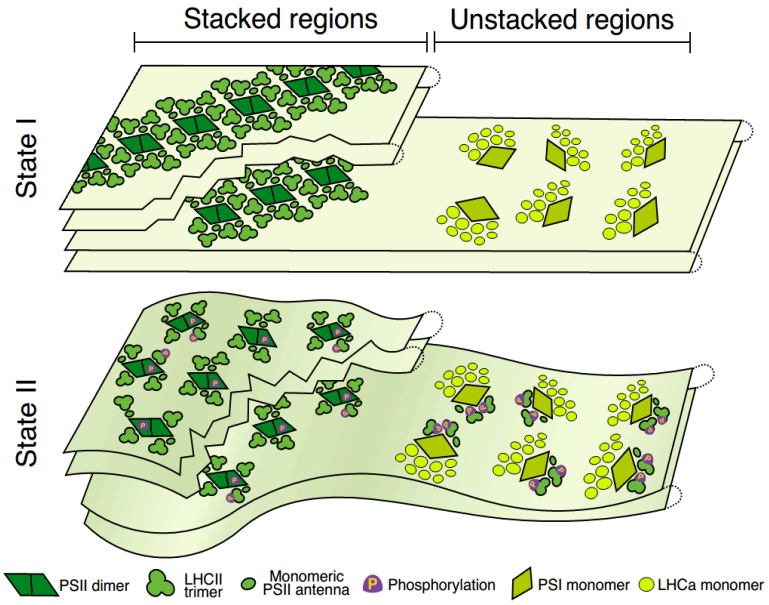
This work was supported by grant 'DYNAMO,' ANR-11-LABX-0011-01 from the French 'Initiative d'Excellence' program.

Energy redistribution or dissipation? How do state transitions regulate energy transfer?

Wojciech J. Nawrocki[1], Stefano Santabarbara[2], Laura Mosebach[1,3], Francis-André Wollman[1*], Fabrice Rappaport[1]

[1]: Institut de Biologie Physico-Chimique, UMR 7141 CNRS-UPMC, 13 rue P. et M. Curie 75005, Paris, France
[2] : Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Via Celoria 26, 20133 Milano, Italy.
[3]: present address: Munster University, Institute of Plant Biology & Biotechnology, D-48143 Muenster, Germany
* wollman@ibpc.fr

Photosynthesis feeds the biosphere in energy by converting sunlight into biologically useful compounds. This catalytic feat involves two photosystems acting in series and powered by Light Harvesting Complexes which increase the flux they sustain by many folds. These complexes are the main targets of the regulatory processes that allow photosynthetic organisms to cope with a broad range of light intensities. In microalgae, which attract considerable biotechnological interest owing to the potentialities offered by their metabolic plasticity, one of such process, State Transitions, has much larger amplitude than in terrestrial plants whereas thermal dissipation of energy, the dominant regulatory mechanism in plants, only takes place after acclimation in microalgae, suggesting distinct and complementary roles for these two processes. Here we show that, at variance with recent reports, in microalgae State Transitions do not dissipate light energy but redistribute it between the two photosystems thereby allowing a well-balanced influx of exciting energy.



This work was supported by grant 'DYNAMO,' ANR-11-LABX-0011-01 from the French 'Initiative d'Excellence' program.

Energy Transfer Between Light Harvesting Complex II and Photosystem I in the Supercomplex Formed During State Transitions.

Stefano Santabarbara[1] and Stefano Caffarri[2]

[1]Consiglio Nazionale delle Ricerche, Istituto di Biofisica, 20133 Milan, Italy
[2]Aix Marseille Université, Laboratoire de Génétique et Biophysique des Plantes, 13009 Marseille, France
*stefano.santabarbara@cnr.it

The excited state dynamics in the PSI-LHCI and PSI-LHCI-LHCII supercomplexes isolated from Arabidopsis have been investigated by time-resolved fluorescence spectroscopy with picosecond temporal resolution. The excited state decay was analysed both in terms of a sum of discrete exponential and in terms of continuous distribution of lifetimes. The analysis indicates the presence of broad amplitude distribution in the 0.4-15 ps lifetime interval, as well as narrower distributions centres at ~35 and ~90 ps. The centres of these lifetime amplitude distributions are only slightly affected by the coupling of LHCII to the PSI-LHCI supercomplex. However, both the width and spectral dependences of the distributions of lifetimes are modified in the ternary complexes. The width of the lifetime amplitude density cluster in the 0.4-15 ps windows broadens further when LHCII is coupled to PSI. Moreover the spectral dependence of the at ~35 and ~90 ps lifetime density clusters blue-shifts because of an increase in amplitude in the 675-685 nm region, which is where LHCII contributes the most . The analysis of the results in terms of spectral-kinetic modelling indicates that energy transfer from LHCII to the bulk of PSI antenna occurs with average macroscopic transfer rates in the 30-60 ns-1 interval. This values is of the same order of magnitude of the estimated average energy transfer rate from the low energy spectral forms of LHCI to the bulk of PSI antenna (15-40 ns-1) but slower than the transfer from the bulk antenna of PSI to the reaction centre (>150 ns-1), implying a relatively small kinetics bottleneck for energy transfer from LHCII. Nevertheless, the kinetic limitation imposed by excited state diffusion has little impact on the photochemical quantum efficiency of the supercomplex, differently from what observed for similar increase in antenna size of PSII. The physiological implications in adaptive strategy aimed at optimising photosynthetic performance under limiting light regimes will be discussed.

This work was supported by grant 'DYNAMO,' ANR-11-LABX-0011-01 from the French 'Initiative d'Excellence' program.

Working out a photoanode using photosynthetic algae

Adnan Sayegh[1*], Manon Guille Collignon[1*], Jérome Delacotte[1], Guillaume Longatte[1], Francis-André Wollman[2], Fabrice Rappaport[2], Frédéric Lemaitre[1*]

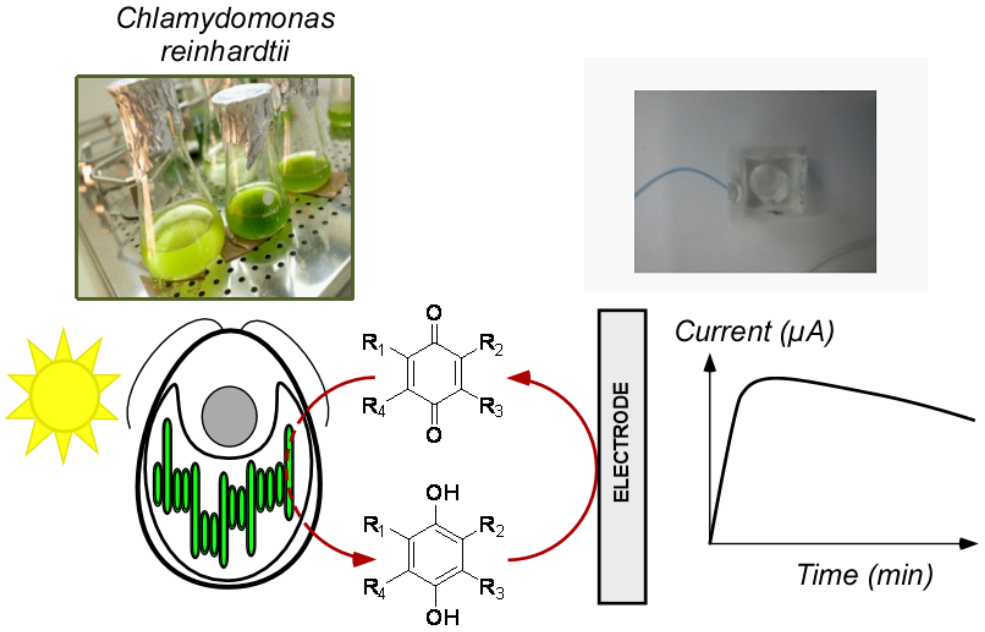
1.Ecole Normale Supérieure—PSL Research University, Département de Chimie, Sorbonne Universités—UPMC Univ Paris 06, CNRS UMR 8640 PASTEUR, 24, rue Lhomond, 75005 Paris, France
2.Laboratoire de physiologie membranaire et moléculaire du chloroplaste, CNRS, UPMC UMR 7141, I.B.P.C., 13 rue Pierre et Marie Curie, 75005 Paris, France
*adnan.sayegh@ens.fr (Adnan Sayegh), manon.guille@ens.fr (Manon Guille-Collignon), frederic.lemaitre@ens.fr (Frédéric Lemaitre)

Nowadays, all over the world, energy is a major concern to societies and research is focusing on finding new sources of clean and sustainable energy.

In this context, photosynthesis may be an appropriate way to meet this objective. Indeed , through the photoconversion of water and CO_2 into sugars and dioxygen, solar light provides the chemical energy required by photosynthetic organisms to maintain their cellular activity.

Taking advantage of photosynthesis to produce a photocurrent requires intercepting electrons exchanged in the oxidation/reduction processes occurring along the photosynthetic chain. This is why we consider here an electrochemical device involving an ITO (Indium Tin Oxide) electrode able to derivate photosynthetic electrons from a population of Chlamydomonas *reinhardtii* algae.

Known as efficient PSII acceptors, quinones were used as exogenous mediators to transfer the electrons from the photosynthetic chain to the electrode surface. Therefore, the resulting photocurrents under illumination, will be commented and discussed in order to understand and define the best appropriate experimental conditions for this bio-solar generator.



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Structure et dynamique des protéines

- **Florian GEORGESCAULD**
  Role of chaperones in mitochondria and chloroplasts protein import

- **Marina KATAVA**
  Computational investigation of the structure and the function of thermophilic proteins at high temperature

- **Marina CASIRAGHI**
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  Structural variability and binding modes of the glioma targeting NFL-TBS.40-63 peptide on tubulin

- **Charles BOU NADER**
  Recognition of tRNA by the human tRNA-Dihydrouridine synthase2: involvement of a double stranded RNA-binding domain

Role of chaperones in mitochondria and chloroplasts protein import

Florian Georgescauld*, Julien Henri, Julia Malaika Maier, Rénette Saint-Fort and Philippe Meyer*

UMR8226, Institut de Biologie Physico-Chimique 75005, Paris
* georgescauld@ibpc.fr and meyer@ibpc.fr

Chloroplasts and mitochondria are organelles of endosymbiotic origin whose functions, such as photosynthesis, respiration or metabolism of amino acids and lipids, are essential for cell survival. Almost all of their proteins are encoded in the nucleus of the cell, synthesized as precursors in the cytosol and post-translationally transported through their membranes. The majority of these proteins are synthesized as preproteins with an amino-terminal signal sequence, addressing the preproteins to the organelles through membrane multi-protein complexes, or translocons, localized on the one hand in the external envelope of chloroplasts (TOC) or internal (TIC) [1,2] and the other hand in the outer (TOM) or internal (TIM) membrane of mitochondria [3,4]. The addressing and translocation of some of these pre-proteins are facilitated by several chaperones (Figure 1). Little structural and functional information is available to understand the mode of recognition and regulation of molecular chaperones by cochaperons receptors associated with translocation machinery. We thus focused our attention on the study of receptors containing TPR motifs (TetatricoPeptide Repeat) and their potential cognate molecular chaperones in Chlamydomonas reinhardtii, Arabidopsis thaliana, Saccharomyces cerevisiae and Homo sapiens.

Upon the 16 different molecular chaperones and membrane receptors targeted, 15 have been cloned, 10 expressed and 9 purified. Finally, we performed an in vitro functional analysis on three chaperone/receptor couples and crystallized some of these receptors. We were able to show that Toc64, Tic40 and Tom34 modulate the activity of their cognate chaperones. These preliminary results suggest a model where chaperone activity modulated by receptors associated with the translocation apparatus could facilitate the release or the capture of preproteins during the translocation.

The structural and functional information provided by this project participates in clarifying the basic mechanisms of biogenesis and regulation of the energy transducing membranes of these organelles that are still poorly understood.

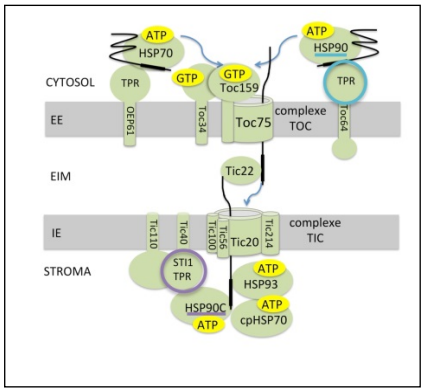

Figure 1 – Structure and function of *A. thaliana* molecular chaperones and cochaperone receptors containing TPR motifs at the chloroplast translocon.

1- Inaba et al. (2008) *Biochem J* **413**,15-28
2- Li et al. (2010) *Annu Rev Plant Biol* **61**,157-180.
3- Dudek et al. (2013) *Biochim Biophys Acta*. **1833**, 274-85.
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This work was supported by grant 'DYNAMO,' ANR-11-LABX-0011-01 from the French 'Initiative d'Excellence' program.

Computational investigation of the structure and the function of thermophilic proteins at high temperature

Marina Katava[1*], Fabio Sterpone[1]

[1]Laboratory of Theoretical Biochemistry, Insistut de Biologie Physico-Chimique, UPR9080, CNRS
*katava@ibpc.fr

Understanding the mechanisms underlying protein stability and function at high temperature is an ongoing challenge. We present multiple computational approaches to tackle related problems on model systems. These include the effect of solvent and crowding to the shift of the melting temperature of Lysozyme as well as the scaling of the molecular vibration approaching the melting in different environments, the relationship among protein flexibility and activity in EF Tu/1alpha G-domain, and the multi-domain Lactate/Malate Dehydrogenases proteins.
Our results are systematically complemented by Neutron Scattering experiments.

Functional modulation of a GPCR conformational landscape in a lipid bilayer

Marina Casiraghi,[1] Marjorie Damian,[2] Ewen Lescop,[3] Elodie Point,[1] Karine Moncoq,[1] Nelly Morellet,[3] Daniel Levy,[4] Jacky Marie,[2] Eric Guittet,[3] Jean-Louis Banères,[2*] Laurent J. Catoire[1*]

[1] Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, UMR7099, CNRS, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France;
[2] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS-Universités Montpellier-ENSCM, Faculté de Pharmacie, 15 Avenue C. Flahault, F-34093 Montpellier, France;
[3] Institut de Chimie de Substances Naturelles, CNRS UPR 2301, Université Paris Sud, 91198 Gif–sur–Yvette, France;
[4] Institut Curie, Centre de Recherche, 75231 Paris, France; UMR 168, UPMC, CNRS, 75231 Paris, France.

*laurent.catoire@ibpc.fr; *jean-louis.baneres@univ-montp1.fr

G protein-coupled receptors (GPCRs) are a large family of integral membrane proteins playing a key role in many biological processes and understanding their signalling mechanism represents one of the main issues in biology. Although we dispose of many 3D crystal structures of GPCRs at atomic resolution, additional biophysical approaches are necessary to characterize their conformational ensemble and the associated kinetic barriers to fully understand the signal transduction process of these receptors.
To this aim we propose an innovative approach to observe GPCRs dynamic conformational landscape and its modulation by ligands and lipids, that are known to play a key role in membrane protein structures and activities [1].
GPCRs kinetic barriers are explored by solution state NMR [2]. We use $^{13}CH_3$ probes, the ideal isotope-labeling scheme dedicated to the study of large proteins [3], immersed in a perdeuterated environment, a mandatory condition to correctly interpret NMR relaxation data when working on large proteins as in our case.
We chose *Escherichia coli* as expression system for its ability to grow in a homogeneously perdeuterated environment. The GPCR expression is directly targeted to *Escherichia coli* inclusion bodies [4]. Once purified, the receptor is folded in amphipols [5],[6] and then transferred to nanometric lipid bilayers (NLBs) [7] to be 100%-active and functional in the conditions of the NMR experiments. Our protocol finally gives rise to up to 6 mg/litre of culture of pure $^{13}CH_3$-u-^{2}H-GPCRs, and the NMR approach allows us to obtain a spectral resolution never reported so far in the GPCR field. Thanks to that, we are able to investigate the complex conformational landscape of our model receptor, the leukotriene B4 receptor (BLT2), that according to our data explores multiple different conformational states as unliganded receptor, including the active state. These unprecedented data that we report for BLT2 will open an avenue for the understanding of the conformational plasticity and regulation of the GPCRs in a lipid environment.

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Use of functionalized amphipols for studying membrane protein supercomplexes by electron microscopy (EM)

Hager Souabni[1], Thomas Perry[2], Francesca Gubellini[2] and Manuela Zoonens[1*]
[1]: UMR 7099, Institut de Biologie Physico-Chimique, 75005 Paris
[2] : UMR3528, Institut Pasteur, G5 Biologie structurale de la secretion bacterienne
*Zoonens@ibpc.fr

Identification of the structure of large protein complexes, such as bacterial secretion systems, plays an important role in the comprehension of pathogenesis mechanisms of micro-organisms. Type IV secretion (T4S) systems are large dynamic nanomachines that transport DNAs and/or proteins through the membranes of bacteria. The complexity and dynamics of the T4S systems and their multiprotein organisation represent serious difficulties for the structural analysis of these systems or even their components. Its architecture as we know it today has been achieved by X-ray crystallography and cryo-EM (1). The structure of its 'core' complex has been obtained using cryo-EM (1,2) revealing that it is composed of three proteins, each present in fourteen copies forming a cage in the bacterial periplasm. Later, the upper portion of the core complex has been crystallized revealing how its subunits are organized at the atomic-scale in this region of the complex (3). More recently, the structure of an assembled T4S complex has been obtained at 20 Å resolution by negative stain-EM (4). This structure showed that T4S forms a highly flexible complex of more than 3 MDa. However, the architecture and relative topological positioning of the rest of the T4S system components, particularly at the inner membrane, are unknown. In order to identify the position of the transmembrane region and help the interpretation of the structural model of these machineries, functionalized amphipols bearing affinity tags can be used.

Amphipols (APols) are small amphipathic polymers designed to keep membrane proteins water-soluble (5, 6). Since recently, APols appear particularly suitable for single-particle EM studies. Labelling tagged-APols that surround the hydrophobic domain of supercomplexes could unveil the position of the membrane region by increasing the electron density of this region. To develop this approach, the core complex of T4S of *E. coli* and the outer membrane lipoprotein CsgG are used as model systems. Functionalized APol bearing biotins (called 'BAPol'), which was developed originally to immobilize membrane protein/BAPol complexes on streptavidin-modified surface (7) is used to trap the protein complexes. Here, we foresee the labelling of BAPol with streptavidin for locating by EM the APol layer surrounding the transmembrane domain of these two large protein complexes. We will generate images of the supercomplexes with crowns of BAPol/streptavidin around their transmembrane areas. The localization of these rings of BAPol/streptavidin will be identified by negative stain to visualize the extra density due to the streptavidin tetramer.

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RNase Y, a new endoribonuclease involved in mRNA processing and degradation in *Bacillus subtilis*

Pierre Hardouin[1*], Béatrice Golinelli-Pimpanneau[1], Harald Putzer[2], Soumaya Laalami [2]

[1]UMR8229, CNRS, Collège de France, 11 Place Marcelin Berthelot, 75005, Paris, France.
[2]FRE3630, CNRS, Institut de Biologie Physico-Chimique, Laboratory of Transcriptional and post-transcriptional controls of gene expression, FRE3630, IBPC, Paris, 75005.
*pierre.hardouin@college-de-france.fr

Messenger RNA decay and processing in bacteria involve a set of various ribonucleases (RNase). In *Bacillus subtilis*, a newly discovered endoribonuclease called RNase Y was shown to play a central role in mRNA decay by catalyzing the initial endonucleolytic cleavage reaction. Its action releases several RNA pieces that are more sensitive to degradation by exoribonucleases. Interestingly, although RNase Y presents a functional homology with *E. coli* RNase E, it does not bear sequence similarity with it and no known structural homolog has been found yet[1,2].
As *Bacillus subtilis* is a model of Gram positive bacteria, which includes several human pathogens (such as *Listeria, Staphylococcus, Streptococcus, Clostridium* species), understanding the mechanism and structure/function of this enzyme may help to design specific inhibitors with antibiotic properties.
The main project consists in producing and crystallizing various constructs of the protein, with the goal to solve their structure by X-ray crystallography. The protein contains an intrinsically disordered domain at the N-terminus, which represents an important part (~30% of the sequence), which is a challenge for crystallography[3]. To address this structural study, we thus used three different strategies. The first one consists in crystallizing RNase Y fused to a maltose binding protein (MBP) optimized for crystallization. The second one is to co-crystallize the protein with the Fragment antigen-binding (Fab) of an antibody directed against RNase Y. The aim is to “freeze” the protein in a specific conformation that helps the crystallization process. Finally, the third strategy aims at gaining insight into the catalytic mechanism by crystallizing RNase Y fragments that contain only the catalytic domain.

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Functional investigation of the MexA / MexB / OprM multidrug efflux pump from *Pseudomonas aeruginosa*

Alice Verchère[1,3], Manuela Dezi[1,4], Yvette Ntsogo[1], Laetitia Daury[2], Vladimir Adrien[3], Gilles Phan[1], Olivier Lambert[2], Isabelle Broutin[1], **Martin Picard**[1,5]*

[1]Laboratoire de Cristallographie et RMN Biologiques, UMR 8015, CNRS, Université Paris Descartes, Faculté de Pharmacie de Paris, 4 Avenue de l'Observatoire, 75006 Paris, France. [2]CNRS, CBMN UMR 5248, Pessac F-33600, France. [3]Laboratoire de physique statistique de l'Ecole Normale Supérieure, UMR 8550, CNRS, Université Pierre et Marie Curie, 24 rue Lhomond, 75005 Paris, France. [3]Present address: Department of Biochemistry, Weill Cornell Medical College, New York, New York 10065, USA. [4]Present address: Institut de minéralogie, de physique des matériaux et de cosmochimie, UMR 7590, Université Pierre et Marie Curie, 4 place Jussieu, 75005 Paris, France. [5]Present address: Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, CNRS UMR 7099, Institut de Biologie Physico-Chimique (IBPC), 13, rue Pierre et Marie Curie 75005 Paris France

* martin.picard@ibpc.fr

Among the various mechanisms developed by Gram-negative bacteria to counter to the effect of antibiotics, active efflux is on the front line. In *Pseudomonas aeruginosa*, the constitutive efflux pump is organized as a multicomponent system where MexB, located in the inner membrane, works in conjunction with MexA, a periplasmic protein, and OprM, an outer membrane protein. MexB acts as a proton motive force-dependent pump with broad substrate specificity. We have developed innovative *in vitro* procedures allowing to monitor the transport and the assembly of the efflux pump.

We first present a procedure where the protein partners are reconstituted into respective proteoliposomes that allow mimicking the two-membrane architecture of a Gram negative bacteria[1]. By use of relevant fluorescent probes, we monitor transport of ethidium bromide through the reconstituted pump. We show that OprM needs to interact with MexA and MexB in order to open, a result in accordance with previous models, and that MexB activity is accelerated when the pump is assembled.

We have also designed a new test that allows investigating the assembly state of the pump[2]. The method relies on the streptavidin-mediated pull-down of OprM proteoliposomes upon interaction with MexAB proteoliposomes containing a biotin function carried by lipids. We give clear evidence for the importance of MexA in promoting and stabilizing the assembly of the MexAB-OprM complex. In addition, we have investigated the effect of the role of the lipid anchor of MexA as well as the role of the proton motive force on the assembly and disassembly of the efflux pump.

Finally, we present a single-particle analysis by electron microscopy of the native efflux pump after its reconstitution in a lipid nanodisc system[3].

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Hydrodynamic effects on Amyloid-β aggregation

Mara Chiricotto[1*], Simone Melchionna[2], Philippe Derreumaux[3], and Fabio Sterpone[1]

[1]Laboratoire de Biochimie Theorique, IBPC, CNRS UPR9080, 75005, Paris, France
[2]Institute for Complex System, Consiglio Nazionale delle Ricerche, Rome, Italy
[3]IUF, Institut Universitaire de France, Boulevard Saint Michel, 75005 Paris, France

*chiricotto@ibpc.fr

The self-assembly of misfolded amyloid-β (Aβ) proteins into insoluble fibrils is strongly linked to the pathogenesis of Alzheimer's disease (AD). The development of new drugs capable to inhibit fibril formation requires the understanding of the mechanism leading to the formation, in the early stages, of metastable oligomers which are the main neurotoxic species.

Our investigation enlighten the role of hydrodynamic interactions (HI) in the kinetics of β-amyloidogenesis, interactions which are essential for modelling such processes occurring in highly crowed environments like cells[1]. Our approach is based on a new multi-scale and multi-physics method that couples Lattice Boltzmann and Molecular Dynamics (LBMD) techniques[2,3]. In our scheme solvent mediated interactions are included naturally. For the molecular system $A\beta_{(16-22)}$ we adopt the high resolution coarse grained model OPEP (Optimized Potential for Efficient Protein structure prediction)[4,5] , developed in our laboratory. For the first time, we have performed quasi-all-atoms simulations for very large systems containing thousands of $A\beta_{(16-22)}$ peptides and approaching experimental concentration.

After the correct tuning of the key parameter of our coupling in order to obtain the experimental diffusivity of $A\beta_{(16-22)}$ monomer and small oligomers, we have demonstrated that HI speed up the aggregation process. A detailed characterisation of the fluctuating clusters is presented in term of their size and the structural organisation of the peptides.The methodology is currently employed to study the aggregation of mixture $A\beta_{(16-22)}$ and $Tau_{(275-280)}$, being also the latter implicated in Alzheimer's disease.

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Structural variability and binding modes of the glioma targeting NFL-TBS.40-63 peptide on tubulin

Yoann Laurin[1], Philippe Savarin[2], Charles Robert[1], Masayuki Takahashi[3], Joel Eyer[4], Chantal Prevost[1], Sophie Sacquin-Mora[1]*.

1. Laboratoire de Biochimie Théorique, UPR 9080 CNRS Institut de Biologie Physico-Chimique,13 rue Pierre et Marie Curie, 75005 Paris, France
2. Université Paris 13, Sorbonne Paris Cité, CSPBAT, UMR 7244 CNRS, 74 rue Marcel Cachin, 93017 Bobigny, France
3. School of Bioscience and Biotechnology, Tokyo Institue of Technology 2-12-1-M6-14 Ookayama, Meguro-ku, Tokyo 152-8550 Japan
4. Laboratoire de Neurobiologie & Transgenèse, UPRES EA 3143, INSERM, Centre Hospitalier Universitaire, Angers, France
* sacquin@ibpc.fr

NFL-TBS.40-63 is a 24 amino acid peptide corresponding to the tubulin-binding site located on the light neurofilament subunit which selectively enters glioblastoma cells, where it disrupts their microtubule network and inhibits their proliferation[1-3]. We investigated its structural variability and binding modes on a tubulin heterodimer using a combination of NMR experiments, docking and molecular dynamics simulations. Our results show that, while lacking a stable structure, the peptide binds specifically on a single site located near the β-tubulin C-terminal end[4]. This C-terminal end, intrinsically unstructured, don't have any resolved crystal structure. We decided to model complete tubulin heterodimer to study the dynamic behavior and potential interaction of the NFL-TBS.40-63 peptide with different tubulin isotypes of the β-monomer. The isotypes modeled are dimer α1-β1 and α1-β3 tubulins giving us insight of the differences in structure, interaction with the other parts of the tubulin and potentially with the peptide of the newly modeled C-termini.

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Recognition of tRNA by the human tRNA-Dihydrouridine synthase2: involvement of a double stranded RNA-binding domain

Charles Bou Nader[1], Pierre Barraud[2], Ludovic Pecqueur[1], Damien Bergeon[3], Carine Tisne[2], Marc Fontecave[1] and Djemel Hamdane[1*]

[1]*Laboratoire de Chimie des Processus Biologiques, CNRS-FRE 3488, Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05*
[2]*Laboratoire de Cristallographie et RMN Biologiques, CNRS, Paris Sorbonne Cité, 4 avenue de l'Observatoire, Paris, 75006*
[3]*Sorbonne Universités, UPMC Univ. Paris 06, UMR8256, Biology of Aging and Adaptation, F-75005 Paris*
*djemel.hamdane@college-de-france.fr

The human dihydrouridine synthase 2 (hDus2) is a flavoenzyme that catalyzes the specific reduction of uridine 20 in several tRNAs. This enzyme was overexpressed in non-small cell lung cancers (NSCLC) [1,2] and its selective suppression by an siRNA inhibited NSCLC growth making hDus2 an interesting therapeutic target.

Recent bioinformatics studies suggested a modular organization of hDus2 consisting of an Nt Dus domain and a Ct double stranded RNA-binding domain (dsRBD) [3]. This architecture was recently confirmed experimentally in our lab by resolving the crystal structures of these isolated subdomains (2.7 and 1.7 Å resolution for the Dus and dsRBD domains, respectively). A clear electron density was assigned to a redox FMN cofactor in the catalytic center located within the Dus domain that adopts a TIM Barrel fold.

In vitro enzymatic activities and RNA gel shift assay indicate that the overall binding of tRNA is mainly conducted by the dsRBD [4]. To our knowledge, this is the first reported case of a dsRBD specifically recognizing tRNA and the first description of a tRNA-modifying enzyme having a dsRBD.

Based on NMR, small angle X-ray scattering and mutagenesis studies, we propose for the first time a 3D structural model of the tRNA-hDus2 complex and a molecular mechanism for tRNA recognition. Our results shed light on a novel cooperative recognition mode involving the dsRBD that was acquired recently in higher eukaryotes and they explain how this novel tRNA binding mechanism evolved among the large dihydrouridine synthase family.

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Métabolisme et biologie des ARNs

- **Liuba MAZZANTI**
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  The transcriptomic landscape of the Chlamydomonas chloroplast as revealed by sRNA-Seq and WTSS

Integrative coarse–grained RNA modeling for small–angle X–ray scattering

Liuba Mazzanti[1]*, Bertrand Caron[1], Philippe Derreumaux[1,2], Samuela Pasquali[1]

[1] LBT, UPR 9080 CNRS, Université Paris Diderot, Sorbonne, Paris Cité, IBPC
[2] IUF

* mazzanti@ibpc.fr

The coarse-grained model HiRE-RNA[1] can predict complex structures for large RNA molecules, given only their sequence. I will show how this model has been coupled to SAXS experimental data, mainly aiming at a faster convergence to the conformation corresponding to the data. SAXS intensity profiles for coarse-grained systems are constructed integrating the atomistic form factors, and they are shown to be characterized by a mean correction, with periodicity related to the coarse-grain scale. On the one hand, the SAXS bias is introduced at the level of the energy, through a scoring function comparing the coarse-grained profile to the target profile (experimental or theoretical). On the other hand, a force is derived from the SAXS energy term, and is applied to the molecule to drive it towards the target structure. I will present the results for molecules of different sizes (including applications to bistable molecules, for which conformations of similar energies but different shapes are present, making them particularly interesting as benchmarks). At a preliminary level, comparison between SAXS-biased and unbiased simulations shows that the bias toward a specific conformation favors its formation. Moreover, I will illustrate a tool developed in order to analyze coarse-grained structures and molecular dynamics simulations trajectories considering their 2D structure and topology. This tool allows for the systematic network graph representation of 2D structures clusters, making the 2D and topological properties of the structures present in a molecular dynamic simulation or in any set (e.g. obtained from path sampling methods) easy to read.

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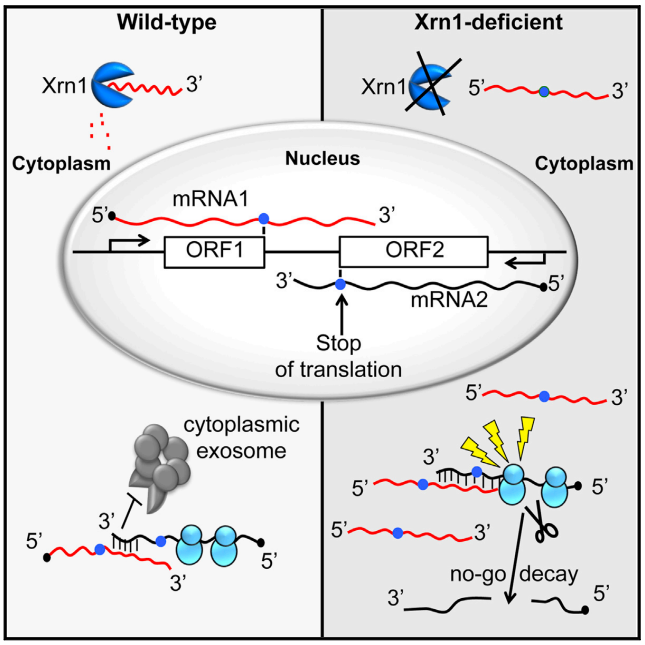
Cytoplasmic fate of sense-antisense mRNA pairs

Albertas Navickas[1,4], Flore Sinturel[1,3,4], Maxime Wery[2], Marc Descrimes[2], Antonin Morillon[2], Claire Torchet[1], and Lionel Benard[1]*

[1]Sorbonne Universités, UPMC Paris 06, CNRS UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, Paris, France
[2]Institut Curie, PSL Research University, CNRS UMR3244, Université Pierre et Marie Curie, Paris, France
[3]Present address: Department of Molecular Biology, Sciences III, University of Geneva, Geneva, Switzerland
[4]Co-first author
*lionel.benard@ibpc.fr

Transcriptome analyses have revealed that convergent gene transcription can produce many 3'-overlapping mRNAs in diverse organisms[1,2,3]. Few studies have examined the fate of 3'-complementary mRNAs in double-stranded RNA-dependent nuclear phenomena[4], and nothing is known about the cytoplasmic destiny of 3'-overlapping messengers or their impact on gene expression. We demonstrate that the complementary tails of 3'-overlapping mRNAs can interact in the cytoplasm and promote post-transcriptional regulatory events including no-go decay[5] (NGD) in *Saccharomyces cerevisiae*. Upon RNAi reconstitution in *S. cerevisiae*[6], genome-wide experiments confirm that these messenger-interacting mRNAs (mimRNAs) form RNA duplexes in wild-type cells and thus have potential roles in modulating the mRNA levels of their convergent gene pattern under different growth conditions. We show that the posttranscriptional fate of hundreds of mimRNAs is controlled by Xrn1, revealing the extent to which this conserved 5'-3' cytoplasmic exoribonuclease plays an unexpected but key role in the post-transcriptional control of convergent gene expression.



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Regulating bacterial gene expression with RNAs: the control of envelope composition and iron uptake

Jonathan Jagodnik, Claude Chiaruttini and Maude Guillier*

Laboratory of "Microbial Gene Expression", CNRS UMR8261,
Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris
*maude.guillier@ibpc.fr

Small regulatory RNAs (sRNAs) are now recognized as major actors of control of gene expression in all kingdoms of life. In bacteria, a large class of these molecules pair with target-mRNAs via imperfect duplexes and control their expression at the post-transcriptional level. These target-genes are involved in a wide variety of cellular functions, ranging from quorum-sensing to iron homeostasis. Notably, synthesis of many membrane proteins is regulated by sRNAs. This is true for instance for the FepA outer membrane protein which plays a major role in iron acquisition as it is the receptor for enterobactin, the siderophore that has the strongest affinity for iron.

OmrA and OmrB are two previously identified sRNAs that repress FepA synthesis (1,2). In most cases, negatively acting sRNAs pair to target-mRNAs in the vicinity of the translation initiation region and thereby inhibit ribosome binding. The situation is different in this case as OmrA/B interact with *fepA* mRNA within the ORF, and presumably too far from the Shine-Dalgarno region to prevent ribosome binding (3). Surprisingly however, *in vitro* experiments strongly suggest that pairing of either OmrA/B specifically inhibits ribosome binding to *fepA* mRNA, even when OmrA/B are restricted to their pairing region with *fepA*. Combined with structural probing of *fepA* mRNA, these data suggest the existence of a conformational switch of *fepA* mRNA that sequesters the Shine-Dalgarno region upon interaction with either sRNA.

Moreover, we discovered that yet two other base-pairing sRNAs, which respond to different input signals than OmrA/B, repress FepA synthesis. Interestingly, each one of the four sRNAs regulating *fepA* acts independently from the others. Furthermore, our results indicate that each of these two additional sRNAs acts on a different region of *fepA* mRNA, and involves different regulatory mechanisms than OmrA/B.

This work highlights the great versatility of regulatory RNAs that allow integration of multiple stimuli to precisely control gene expression and that can target different regions of a single mRNA by acting via different regulatory mechanisms.

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The transcriptomic landscape of the Chlamydomonas chloroplast as revealed by sRNA-Seq and WTSS.

Marina Cavaiuolo[1]*, Yves Choquet[1], Richard Kuras[1], Francis-André Wollman[1], Olivier Vallon[1]

[1]Unité Mixte de Recherche 7141, CNRS/UPMC, Institut de Biologie Physico-Chimique, F-75005 Paris, France
* cavaiuolo@ibpc.fr

Regulation of gene expression is a dynamic process controlled by a number of mechanisms going from RNA synthesis to degradation. In the chloroplast of *Chlamydomonas reinhardtii* regulation of gene expression is mainly post-transcriptional. In particular, it involves gene specific stabilizing factors (M-factors) and ribonucleases that cooperate in maintaining the steady state levels of RNA abundance from mRNA maturation and stabilization to decay [1-3].

We used directional whole transcriptome shotgun sequencing (WTSS) to explore both sense and antisense transcription of the chloroplast genome. We combined it with small RNA sequencing (sRNA-Seq) of short RNAs (< 50-nt) which we consider result from RNA degradation rather than specific transcription.

By sRNA-Seq, we found highly abundant short RNAs at the mature 5' end of almost all chloroplast mRNAs. They correspond to "footprints" of M-factors [4-5], revealing protection of the 5' end against exonucleolytic attack by binding of the protein. They are absent or severely reduced in specific M-factor mutants. Enzymatic pyrophosphohydrolysis allowed us to distinguish those that derive from primary 5' tri-phosphorylated transcripts and from processed mRNAs. For the first time, sRNA sequencing has thus allowed high resolution mapping of 5' ends in a chloroplast.

In addition, short RNAs were also found that mapped to UTRs and coding sequences. Whereas WTSS indicated that for most genes coverage on the coding strand is about 100-1000 higher than on the antisense strand, the sRNAs showed comparable coverage on both strands. When cells were treated with lincomycin or chloramphenicol, respectively inhibitors of chloroplast translation initiation and elongation, the abundance of antisense short RNAs increased. We propose that this results from the processing of double-stranded RNA generated through the pairing of the antisense RNA with the much more abundant sense RNA, normally prevented by the traveling of the ribosomes. In addition, chloramphenicol treatment, by freezing ribosomes on the CDS, led to the accumulation of 32-34 nt sRNAs that we interpret as ribosome footprints. In contrast, when chloroplast transcription was inhibited with rifampicin, antisense short RNAs disappeared more rapidly than sense short RNAs. These results suggest that antisense transcripts, whether produced from specific promoters or by readthrough from converging transcriptional units, can be degraded by a specific pathway involving base pairing to the sense RNA. Antisense transcripts or their degradation products may function in controlling stability or translation of the sense RNA through base pairing.

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Traduction

- **Caroline LACOUX**
  RNA helicases: their structure, function and properties

- **Gregory BOËL**
  Functional characterization of a new class of protein translation factors belonging to the ABC-F protein family in *E. coli* and cyanobacteria.

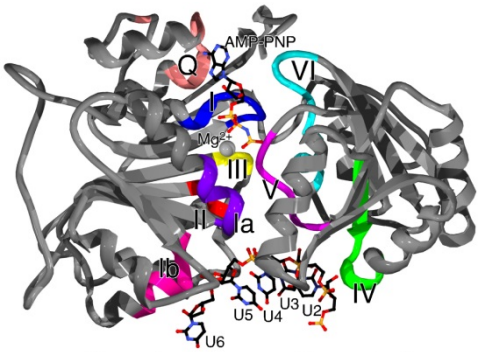
- **Wojciech WIETRZYNSKI**
  Characterization of Rubisco assembly-dependent regulation in *Chlamydomonas reinhardtii*

RNA helicases: their structure, function and properties

Caroline Lacoux[1], Hilal Yeter[1], Josette Banroques[1], Naïma Belgareh-Touzé[2], Thierry Bizebard[1], Vincent Croquette[3], Marc Graille[4], Valérie Heurgué-Hamard[1], Emmeline Huvelle[1], Denis Lafontaine[5], Saurabh Raj[3], & Kyle Tanner*[1]

[1]CNRS UMR8261/Université Paris Diderot Paris 7, IBPC, Paris, France
[2]CNRS/UPMC UMR8226, IBPC, Paris, France
[3]LPS, CNRS UMR8550, ENS, Paris, France
[4]CNRS UMR7654, Ecole Polytechnique, Palaiseau, France
[5]FRS, Université Libre de Bruxelles, Charleroi-Gosselies, Belgique
*kyle.tanner@ibpc.fr

Helicase is a generic term for families of proteins that belong to larger superfamilies (SF) of NTP-dependent proteins involved in the processing, replication, repair, expression and remodeling of nucleic acids complexes. There are a least seven superfamilies of these proteins that use DNA or RNA as substrates. They are characterized by conserved nucleotide binding motifs and core structures consisting of parallel beta-sheets and surrounding helixes that are similar to the solved crystal structure of the bacterial RecA protein, which is involved in homologous recombination. For that reason, the core structures are called RecA-like domains. The majority of the superfamilies contain a single RecA-like domain while SF1 and 2 have two linked domains. The vast majority of the RNA helicases belong to SF2, and to a lesser extent SF1. RNA helicases are ubiquitous proteins found in all three kingdoms of life that are associated with all processes involving RNA, from transcription, processing, transport, translation and to RNA degradation. The yeast *Saccharomyces cerevisiae* contains at least 45 RNA helicases that are often essential and that are generally associated with specific processes involving RNA; complex processing events, such as ribosomal biogenesis and mRNA splicing, require many different helicase proteins.



We are interested in understanding how RNA helicases work, how their specificity and regulation are conferred, and why they play such important roles in the cell. We concentrate on the DEAD-box and DEAH-box proteins, and we typically use yeast and bacteria as model systems. We use *in silico*, genetic, biochemical and enzymatic approaches, and we collaborate with cellular biologists, molecular biologist, crystallographers and biophysicists. In collaboration with Naïma Belgareh-Touzé, we are studying the cellular location of DEAD-box proteins and how they change with growth conditions. In collaboration with Vincent Croquette and Saurabh Raj, we are using magnetic tweezers to better understand the mode of action of these ATP-dependent molecular motors. In collaboration with Marc Graille, and Denis Lafontaine, we are studying the role of the DEAH-box protein Dhr1 in ribosomal biogenesis and RNA methylation. Much of the laboratory work is involved in studying the DEAD-box protein Ded1, which is a functional homolog of a subfamily of proteins involved in cell-cycle and developmental regulation, which includes the human DDX3 proteins that is involved in oncogenesis and in HCV and HIV viral replication. We are interested in identifying the protein partners and RNA substrates of Ded1 to better understand its essential role in the cell.

Functional characterization of a new class of protein translation factors belonging to the ABC-F protein family in *E. coli* and cyanobacteria.

Amin Omairi-Nasser[1], Fares Ousalem[1], Ruben L. Gonzalez, Jr.[2], and John F. Hunt[3], **Grégory Boël**[1]*

[1] CNRS UMR 8261 / Université Paris Diderot, Sorbonne Paris Cité, Institut de Biologie Physico-Chimique, 13-rue Pierre et Marie Curie, Paris, France; [2] Department of Chemistry, Columbia University, New York, NY, USA; [3]Department of Biological Sciences, Columbia University, New York, NY, USA

Protein synthesis uses more than 30% of the energy resources of living organism, therefore this process has to be regulated to maintain efficient use of energy. In *Escherichia coli* the energy-sensing translational throttle A (EttA) achieves part of this regulation[1,2]. We have recently showed that the protein EttA is a translation factor that gates ribosome entry into the elongation cycle in an ADP-to-ATP ratio-dependent manner. Our results suggest that EttA maintains translation initiation complexes in a hibernating state when the ADP-to-ATP ratio is high and allow the translation to restart when ATP cellular concentration increases.

EttA belongs to a large family of protein, the ABC-F protein family that is part of the ABC (ATP-Binding Cassette) superfamily. This superfamily represents the most common molecular architecture used to couple transmembrane transport to the hydrolysis of ATP. They belong to a larger superfamily of proteins containing homologous ABC ATPase domains, which includes numerous soluble-protein families that perform diverse functions unrelated to transmembrane transport. ABC-F proteins comprise the most widespread family of soluble proteins within the ABC superfamily. Four representatives of this ABC-F family are present in *E. coli*, two in cyanobacteria, six in *Chlamydomonas reinhardtii* (including one chloroplastic), five in plants, and three in humans.

We will present our published results on the characterization of the molecular mechanism of EttA but also our preliminary results on the function elucidation of the 3 *E. coli*'s paralogues, namely YbiT, YheS and Uup. These results suggest that all the *E. coli* 's ABC-F proteins act on the protein synthesis apparatus. In parallel we have started to investigate the role of the cyanobacteria ABC-F's proteins. The cyanobacterium *Synechocystis* PCC 6803 has two clearly identifiable ABC-F proteins P74317 and P73759 that contain all the features of an ABC-F protein. As of today nothing is known about their function. In attempt to gain knowledge on their function we have constructed deletion mutants of the corresponding genes. These mutants will allow us to determine if the deletion of the genes affect protein synthesis *in vivo*.

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Characterization of Rubisco assembly-dependent regulation in *Chlamydomonas reinhardtii*

Wojciech Wietrzyński, Francis-André Wollman and Katia Wostrikoff*

Laboratoire de physiologie membranaire et moléculaire du chloroplaste
(CNRS-UPMC UMR7141)
*wostrikoff@ibpc.fr

Playing a pivotal role in the global carbon cycle Ribulose 1,5-biphosphate Carboxylase/Oxygenase (RuBisCO) appears as one of the key enzymes of the photosynthesis-driven life on Earth. Due to its environmental and economic importance it became the object of extensive, bioengineering research. Nonetheless, one of the difficulties standing against this effort is the as yet unraveled mechanism of its assembly.

Synthesis of the RuBisCO requires expression and proper assembly of eight chloroplast encoded Large (LS) and eight nuclear encoded Small (SS) subunits. Owing to the dual genetic origins of its two subunits the stoichiometric formation of RuBisCO in the chloroplast needs to be finely tuned. It has been previously demonstrated that accumulation of LS and SS is dependent coordinated process – as SS is being degraded in the absence of LS, while LS translation is being hampered if SS is not present. Moreover, several proteins: whether assembly factors, chaperonins or gene expression factors have been showcased to act during synthesis and holoenzyme formation. One of these factors is MRL1 – a PPR protein required for the stabilization of the *rbcL* transcript. It has been found in high molecular weight complexes within the chloroplast stroma which might contain other, unknown proteins taking part in LS biogenesis.

Using *Chlamydomonas reinhardtii* as a model alga we are trying to decipher the sophisticated process of RuBisCO assembly with the emphasis on MRL1-dependent LS expression and its autoregulation.

Signalisation

- **Arnaud GAUTIER**
  A genetically encodable fluorescence on/off switch for advanced biomolecular imaging
- **Marcello DE MIA**
  Remodeling of the photosynthetic apparatus under Nitrogen and Sulfur starvation in *Chlamydomonas reinhardtii*
- **Staëlle MAKAMTE**
  The hedgehog signaling pathway: structural and functional studies of proteins Patched and SUFU
- **Zhou XU**
  Two routes to senescence in the absence of telomerase

A genetically encodable fluorescence on/off switch for advanced biomolecular imaging

Arnaud Gautier

École Normale Supérieure – PSL Research University, Department of Chemistry, UMR 8640
PASTEUR, Pôle de Chimie Biophysique, 24 rue Lhomond, F-75005 Paris, France.
arnaud.gautier@ens.fr

Biological imaging is essential for revealing the inner workings of living systems. Among the numerous imaging modalities, light microscopy has revolutionized biological research. Microscopes that enable fluorescence imaging in live cells and animals have been indispensable in our current understanding of biological processes. In addition to advances in optics and detectors, imaging has benefited from the development of molecular tools to observe biomolecules in action. During this presentation, I will present the development of a tunable protein tag dubbed Y-FAST (Yellow Fluorescence-Activating and absorption-Shifting Tag). Y-FAST fluoresces instantaneously upon binding a cell-permeant and non-toxic synthetic fluorogenic dye (so-called fluorogen). A unique fluorogen activation mechanism based on two spectroscopic changes, increase of fluorescence quantum yield and absorption red shift, ensures high selectivity and high contrast. Y-FAST was engineered from the 14-kDa photoactive yellow protein (PYP) by directed evolution. Y-FAST compares favorably to common fluorescent proteins in terms of brightness and photostability and functions in diverse organelles, cells and organisms. Y-FAST distinguishes itself from other tagging systems because fluorogen binding is rapid, highly dynamic and fully reversible. This latter feature makes Y-FAST a genetically encodable fluorescence on/off switch holding great potentials for multiplexing imaging, super-resolution microscopy and biosensing.

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Remodeling of the photosynthetic apparatus under Nitrogen and Sulfur starvation in *Chlamydomonas reinhardtii*

Marcello De Mia[1,2*], Stéphane Lemaire[1*], Francis-André Wollman[2], Yves Choquet[2*]

1: UMR8226, 2: UMR7141, Institut de Biologie Physico-Chimique (IBPC) 75005, Paris
* marcello.demia@ibpc.fr; stephane.lemaire@ibpc.fr; yves.choquet@ibpc.fr

Nitrogen and sulfur starvation lead to photosynthesis inactivation in *Chlamydomonas reinhardtii*, mainly through the remodeling of protein complexes involved in the photosynthetic process. The main targets of the remodeling events are the cytochrome b_6f complex, a key component of the photosynthetic electron transport chain within thylakoid membranes, and the RubisCO holoenzyme, responsible for CO_2 fixation in the stroma. Under both nitrogen and sulfur deprivation, these two protein complexes are selectively lost, albeit with a slightly delayed kinetics in S-deprived conditions. In a detailed analysis of the effects of nitrogen starvation on photosynthesis, we have recently revealed that cytochrome b_6f loss is independent of light/dark conditions but is prevented in cells grown in photoautotrophic conditions or, more generally, when respiration is impaired[1]. Photosynthetic inactivation and cytochrome b_6f disappearance were found to correlate with NO production as the the addition of NO-donors/scavengers affects the kinetics of the process. Together, these observations suggest a role for NO in the remodeling of the photosynthetic apparatus in N-starved cells, although information on the underlying molecular mechanisms are still lacking. Moreover, preliminary data suggest that a comparable remodeling occurs under sulfur starvation[2], raising the possibility that similar mechanisms may be involved in both responses. To explore this possibility and get further insights into the associated mechanisms, here we compare the two types of starvation using a combination of biophysical and biochemical approaches. Considering that NO signaling mainly operates through protein nitrosylation, a reversible post-translational modification of cysteine residues[3], our aim is to explore the nitrosylation proteome of both membrane and soluble proteins under each stress condition. Identification of nitrosylated proteins and of their modified residues should shed light on the similarities and differences between both starvation conditions and give further insights into the molecular mechanisms through which NO allows remodeling of the photosynthetic machinery.

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The hedgehog signaling pathway: structural and functional studies of proteins Patched and SUFU

Staëlle Makamté[1*], Valérie Biou[1]

Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, IBPC, 13 rue Pierre et Marie Curie, 75005 Paris
* makamte@ibpc.fr

The Hedgehog signaling pathway is implicated in cell differentiation during embryogenesis[1]. It is also part of the stem cells maintain during adulthood. Discovered during the 80's by Nüsslein-Volhard and Wieschaus, this pathway has been part of many studies describing its functioning and giving the proteins to which it is associated. The HH pathway is very conserved and its disruption causes many human diseases such as holoprosencephaly and some mutations of proteins in this pathway are associated with cancers[2]. The HH pathway activation occurs when the morphogen hedgehog is fixed on its receptor Patched. This activation leads to the expression of some genes *via* the activation of its transcription factor belonging to the Zn finger transcription factor family Gli in human and Cubitus interruptus in drosophila (CI/Gli). On the other hand, when the pathway is switched off, CI/Gli is phophorylated, partly degraded into a shorter, repressing form and inhibited by another protein, Suppressor of fused (SUFU).

SUFU is a 52 kDa soluble protein organized in α helices and β sheets and whose 3D[3,4] structure has been published in 2013. Atomic emission spectroscopy allows me to determine that drosophila SUFU (dSUFU) fixes Zn and that this fixation increases with the pH. Due to a coloured compound, I have been able to determine that dSUFU has a nanomolar affinity for the Zn. Furthermore, SAXS data obtained dSUFU, human SUFU (hSUFU) and zebrafish SUFU (zSUFU) show that hSUFU and zSUFU present different oligomerization states than dSUFU in solution. As the mutation of some residues in the Nterm of hSUFU is involved in some cancers and the mutation of the two histidines limits the stabilization of dSUFU, these findings can open new insights into SUFU structure and I intend to crystallise dSUFU associated with Zn to confirm the location of this cation in dSUFU.

The Hedgehog signaling pathway also includes the 152 kDa transmembrane protein patched. This protein has 12 transmembrane segments and two large extracellular domains. Biochemistry studies realized by Nakamura et al showed that the two large extracellular domains are sufficient for the binding of its ligand Hedgehog. Moreover, Isabelle Mus-Veteau showed that Patched is involved in anticancer drugs efflux. However, there is no structural study conducted on this protein. In order to structurally study patched, I have designed 2 constructions of the human Patched (hPtc) protein. The first construction is the two extracellular domains attached to the lysozyme T4, the second one is the protein truncated of its N and C terminal domains.

During this past two years of PhD, I have been able to determine that dSUFU has an affinity with Zn in a pH dependant manner. I have initiated studies that will allow structural studies of the transmembrane protein hPtc.

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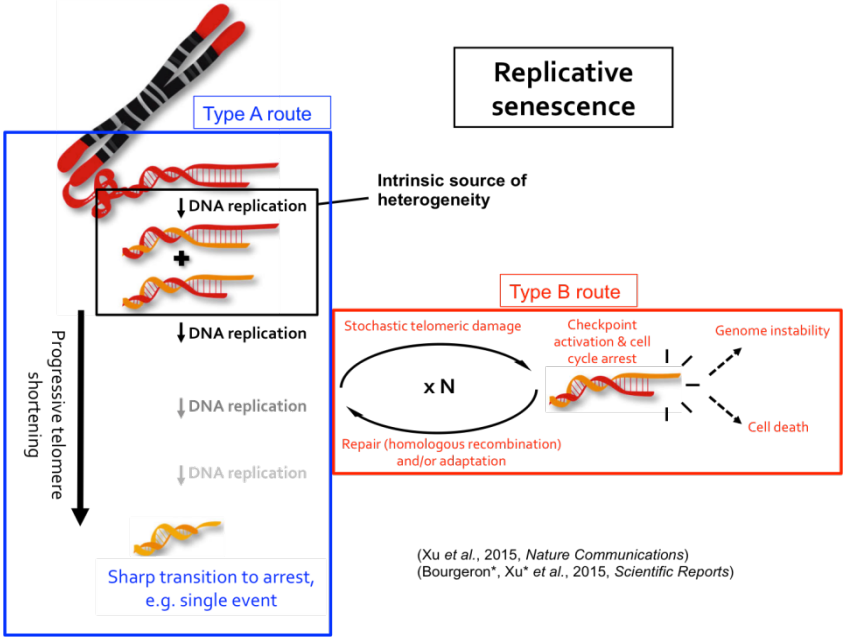
Two routes to senescence in the absence of telomerase

Zhou Xu[1], Thibault Bourgeron[2], Camille Paoletti[3], Steffen Fehrmann[3], Emilie Fallet[1], Marie Doumic[2], Gilles Charvin[3], Maria Teresa Teixeira[1,*]

[1]Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, 75005 Paris, France, ERC-STG-2010-D-END.
[2]INRIA Paris-Rocquencourt, Université Pierre et Marie Curie, Paris, France,
[3]IGBMC, Illkirch, France
*teresa.teixeira@ibpc.fr

Failure to maintain telomeres leads to their progressive erosion at each cell division. This process is heterogeneous but eventually triggers replicative senescence, a pathway shown to protect from unlimited cell proliferation. However, the mechanisms underlying its variability and its dynamics are not characterized. Here, we used a microfluidics-based live-cell imaging assay to investigate replicative senescence in individual *Saccharomyces cerevisiae* cell lineages following telomerase inactivation. We show that most lineages experience an abrupt and irreversible transition from a replicative to an arrested state, contrasting with the idea of a progressive transition. Such a sharp switch is fully consistent with a mathematical model where the first telomere reaching a critical short length triggers senescence onset. Notably, a considerable part of replicative senescence heterogeneity is structurally built in the asymmetrical telomere replication mechanism. However, many lineages also undergo frequent reversible DNA damage checkpoint cell-cycle arrests, beginning soon after telomerase inactivation. Cells with this phenotype persist only at low frequency in bulk cultures, making them undetectable in conventional population-averaged assays. Based on data obtained in *RAD51*, *MEC1* and *POL32* mutant backgrounds, we propose a model where telomere replication fragility, enhanced by telomerase inactivation, initiates both genomic instability and post-senescence survival. These data reveal a cryptic route to senescence and suggest that another source of heterogeneity of senescence onset consists of stochastic telomere damages that require telomerase or homologous recombination for repair.



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Régulation et prolifération membranaire

- **Dror WARSCHAWSKI**
  Watching lipids directly in the cell: fluorescence microscopy and solid-state NMR
- **Federica ANGIUS**
  Molecular basis of membrane protein production and internal membranes proliferation in *Escherichia coli*
- **Naima BELGAREH-TOUZÉ**
  Novel implications of the ubiquitin conjugation system in yeast mitochondrial dynamics and mitophagy
- **Dario DE VECCHIS**
  Mitochondrial membrane fusion: computational modelling of mitofusins

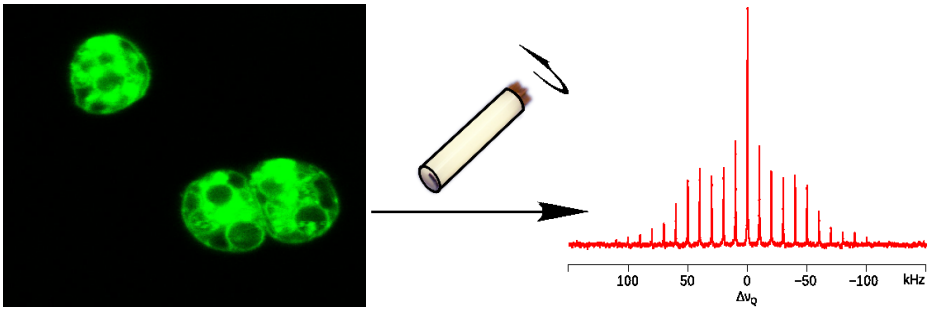
Watching lipids directly in the cell:

fluorescence microscopy and solid-state NMR

Xavier L. Warnet[1], Marwa Laadhari[2], Oana Ilioaia[1], Alexandre A. Arnold[2], Francesca Zito[1], Isabelle Marcotte[2] and **Dror E. Warschawski**[1,2,*]

[1]UMR 7099, CNRS-Université Paris Diderot, Institut de Biologie Physico-Chimique, Paris, France and [2]Department of Chemistry, Université du Québec à Montreéal, Montréal, Québec, Canada
*dror.warschawski@uqam.ca

Our study aims at applying molecular-based approaches to whole cells in order, for example, to be able to observe membrane molecules by NMR[1,2]. We will show here that we can study bacterial membrane lipids by ^{2}H solid-state NMR, in conditions that are compatible with cell survival[3]. Beside structural and dynamic studies, we suggest that measuring the membrane fluidity by ^{2}H solid-state NMR can be used as a diagnostic tool for cellular health. We now wish to apply this approach to micro-algae for which we first have to optimize the labeling of the membrane lipids. With this objective in mind, we will show our first results obtained by fluorescence microscopy.



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Molecular basis of membrane protein production and internal membranes proliferation in *Escherichia coli*

Federica Angius[1], Oana Ilioaia[1], Gerardo Carranza Ferrer[2], Audrey Solgadi[3], Christiane Bouchier[4], Jean-Yves Coppee[4], Ignacio Arechaga[2], Marc Uzan[1] and Bruno Miroux[1*]

[1] Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, Institut de Biologie Physico-Chimique, CNRS, Univ Paris Diderot, Sorbonne Paris Cité, PSL research university, France
[2] Departamento de Biologìa Molecular, Universidad de Cantabria e Instituto de Biomedicina y Biotecnologìa de Cantabria (IBBTEC), UC-CSIC-SODERCAN, Santander, Spain
[3] Institut Paris-Saclay d'Innovation Thérapeutique IPSIT, Service d'Analyse des Médicaments et Métabolites, Faculté de pharmacie - Université Paris-Sud, Chatenay-Malabry, France
[4] Institut Pasteur, Paris, France
**Bruno Miroux, miroux@ibpc.fr

The most successful *E.coli* expression system used to produce membrane proteins for structural studies is based on the T7 RNA polymerase [1]. However, the major drawbacks of this system is the over-transcription of the target gene due to the T7 RNA polymerase transcription activity that is over ten times faster than the *E.coli* enzyme. Since the isolation of spontaneous mutants, namely C41 λ(DE3) and C43 λ(DE3) [2] and the identification of their mutation in the genome, it becomes clear that reducing the amount of the T7 RNA polymerase level removes the toxicity associated with the expression of some membrane protein [3,4]. Also, some membrane proteins require very low rate of transcription to be correctly folded in the E. coli membrane. The first objective of my PhD was to extend the promoter strength coverage of the T7 based expression system. We used genetic and genomic approaches to isolate and characterize new bacterial strains (Angius et al., 2016) in which the level of T7 RNA polymerase is differently regulated than in the existing hosts. A second objective is to understand internal membrane proliferation in *E. coli*. It has been shown that, for instance, over-expression of AtpF of *E. coli* F_1F_o ATP synthase is accompanied by the proliferation of intracellular membranes enriched in cardiolipids [5]. To understand metabolic pathways involved in membrane biogenesis, proliferation and organization, we have used a RNA sequencing approach at several time points upon over-expression of AtpF in C43 λ(DE3). On the other hand, in collaboration with Gerardo Carranza Ferrer and Ignacio Arechaga of University of Cantabria (Spain) we studied C43 λ(DE3) cls mutants, in which the cardiolipids genes A, B and C are deleted, to test how cardiolipids participate to the structuration of intracellular membranes.

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Novel implications of the ubiquitin conjugation system in yeast mitochondrial dynamics and mitophagy.

Naïma Belgareh-Touzé[1]*, Laetitia Cavellini[1] & Mickaël Cohen[1]

1- Sorbonne Universités, UPMC, CNRS, UMR8226, IBPC, 75005 Paris, France.
*belgareh@ibpc.fr

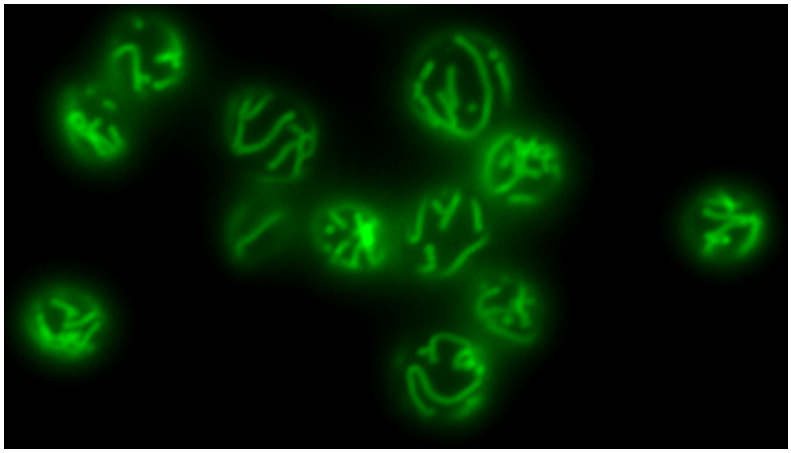
Mitochondria are highly dynamic organelles whose morphology is conditioned by a constant equilibrium between fission and fusion events. These processes are essential to control the mitochondrial morphology and are also crucial for all mitochondrial functions including oxidative phosphorylation and apoptosis. Consequently, defects in mitochondrial dynamics are associated with numerous pathologies and severe neurodegenerative syndromes.

Mitochondrial fusion and fission involve proteins that belong to the super-family of Dynamin-Related Proteins (DRPs). While fission is promoted by Drp1, fusions of mitochondrial outer and inner membranes are mediated by two distinct DRPs: the mitofusins and OPA1, respectively.

In mammalian cells, ubiquitin has emerged as a key regulator of mitochondrial dynamics with mitofusins and Drp1 being targeted for ubiquitylation by distinct ubiquitin ligases including MITOL/March5, MULAN, Huewe1 and Parkin. Ubiquitylation regulates the protein levels of DRPs but most importantly plays a general role in mitochondrial quality control and mitophagy.

In yeast, the only DRP known to be ubiquitylated is the mitofusin Fzo1. We have previously shown that Fzo1 is ubiquitylated by Mdm30, an F-box protein of the SCF (Skp1-Cullin-F-box) ubiquitin ligase complex. The SCF^{Mdm30} ligase promotes ubiquitylation and degradation of Fzo1 to facilitate fusion of outer membranes.

Here, we will present unpublished observations focusing on the previously unappreciated involvement of an ubiquitin-ligase, distinct from Mdm30, in yeast mitochondrial dynamics and mitophagy.



Yeast mitochondria network

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Mitochondrial membrane fusion: computational modelling of mitofusins

Dario De Vecchis[1], Marc Baaden[1], Laetitia Cavellini[2], Mickael Cohen[2], Jérôme Hénin[1], Antoine Taly[1]*

[1]Laboratory of Theoretical Biochemistry LBT 9080, CNRS, Univ Paris Diderot, IBPC, 13 rue Pierre et Marie Curie, 75005 Paris – France.
[2]Laboratory of Molecular and Cellular Biology of Eukaryotes UMR 8226, CNRS, IBPC, 13 rue Pierre et Marie Curie, 75005 Paris – France.

*taly@ibpc.fr

Fzo1, a large GTPase of the Dynamin-Related Proteins superfamily, is a key component in mitochondrial outer membrane fusion and is required for maintain mitochondrial dynamics and morphology. The protein is anchored to the outer membrane by two transmembrane segments and its N-terminal GTPase domain and C-terminal are exposed to the cytosol. Recent data indicate that the Fzo1 GTPase domain would induce a conformational change concurrently with mitochondrial tethering, thus promoting membrane fusion[1]. We investigate the structure and dynamics of Fzo1 through molecular modelling and all-atom simulation in a model mixed lipid bilayer, closely linked to experiments. Our structural model integrates information from several template structures, experimental knowledge, as well as *ab initio* models of the transmembrane segments that are unique to Fzo1. The model is validated experimentally through charge swap mutations across predicted salt bridges and a series of N-terminal truncation mutants indicates that this region is dispensable for function. Our approach unravels hinges domains involved in the conformational change and identified critical residues required for protein stability. Moreover several point mutation found to disrupt the architecture of the protein are located in the coiled-coil domain which has been shown fundamental for the protein. Finally, we dissected key residues in protein-GDP interaction providing fundamental insights about molecular mechanisms by which mitofusins catalyze membrane fusion.

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De la bactérie à l'organelle, évolution

- **Emmanuelle BOUVERET**
  Characterization of the ppGpp synthesis and degradation enzymes of *Chlamydomonas reinhardtii*
- **Magali LEROY**
  Role of a new endoribonuclease YacP in selective mRNA turnover in *Bacillus subtilis*
- **Anna LIPONSKA**
  RNase J of *Chlamydomonas reinhardtii*

Characterization of the ppGpp synthesis and degradation enzymes of *Chlamydomonas reinhardtii*

Emmanuelle Bouveret,

LISM, UMR7255, CNRS, 31 chemin Joseph Aiguier, 13009 Marseille

ppGpp is a hyperphosphorylated nucleotide derived from guanosine, which plays a central role in bacterial growth control. Genes coding for homologs of bacterial ppGpp synthesis enzymes are also found in the genomes of photosynthetic eucaryotes. The enzymes are predicted to be localized in the chloroplast, and the presence of ppGpp has been confirmed in organisms such as *Arabidopsis* or *Nicotiana*. ppGpp might control bacterial-like processes in the chloroplast, such as genome expression or fatty acid biosynthesis. It might therefore also participate to the coordination of the biogenesis and assembly of photosynthetic membranes, in response to various stresses. Our goal is to characterize the 3 genes of *Chlamydomonas reinhardtii* that contain the specific domain of ppGpp synthesis. Using complementation experiments in *E. coli*, we showed that each of these 3 enzymes is capable of ppGpp synthesis. However, we still miss the enzyme responsible for the essential ppGpp degradation activity. Using *E. coli* as a host, the full proteins or domains have been purified. They will be used for *in vitro* studies and for the production of antibodies in order to study the expression and the localization of these enzymes in *Chlamydomonas*. Finally, mutants of *Chlamydomonas* have been recently released, which will allow us to test the role of the ppGpp synthesis genes in the physiology of *Chlamydomonas*.

This work was supported by grant 'DYNAMO,' ANR-11-LABX-0011-01 from the French 'Initiative d'Excellence' program.

Role of a new endoribonuclease YacP in selective mRNA turnover in *Bacillus subtilis*

Magali Leroy[1], Jean Yves Coppée[2] and Ciarán Condon[1*]

[1]CNRS FRE 3630 (affiliated with Univ. Paris Diderot, Sorbonne Paris Cité), Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France, [2]Plate-forme Transcriptome et Epigenome, Genopole - Institut Pasteur, 25-28 rue du Dr. Roux, 75015 Paris, France
** Condon@IBPC.fr*

The Gram-positive model organism *B. subtilis* currently has 19 known ribonucleases, consisting of 8 exoribonucleases and 11 enzymes that cleave RNA endonucleolytically[1]. Some are relatively specialised, such as RNase M5 in 5S rRNA maturation[2] and RNase P and RNase Z in tRNA maturation[3], while others have both stable RNA and mRNA substrates. Despite the large number of RNases identified in *B. subtilis,* there remain processed RNAs for which the enzymes are not yet known, and orphan enzymes whose substrates have not been identified.

We have characterized an orphan enzyme of *B. subtilis* called YacP. This enzyme was first predicted to be a ribonuclease related to the PIN-family (PilT N-terminal) of RNases[4]. Bioinformatics analysis showed that the *yacP* gene is well-conserved, but largely confined to the Firmicutes and the Cyanobacteria, yet it is also found in the chloroplasts of many higher plants. In *B. subtilis*, *yacP* is found in an operon with genes encoding three aminoacyl-tRNA synthetases, a 23S rRNA processing enzyme and an rRNA/tRNA modifying enzyme, and immediately upstream of another large operon encoding ribosomal proteins and enzymes of the core transcription apparatus. Thus YacP potentially plays a role in translation in this organism.

Comparative transciptome analyses of a wild-type strain and a *ΔyacP* mutant by RNAseq allowed for the identification of potential targets of YacP. Among these, we focused on two key transcripts that were stabilized in the absence of YacP. The first encodes a multidrug efflux transporter (*bmrCD*) and the other encodes several putative small peptides, at least one of which (YrzI) is highly toxic to *B. subtilis*. Interestingly, these two transcripts are among the most sensitive in *B. subtilis* to chloramphenicol[5], an inhibitor of translation elongation. Our studies have allowed us to hypothesize as to the mechanism of *yrzI* mRNA stabilization in the presence of chloramphenicol. We have also resolved the role of YacP in the turnover of the *yrzI* mRNA, identifying all key degradation intermediates, other RNases involved and precisely mapped the YacP cleavage site *in vivo*. Overall, our results suggest that the YrzI/YacP pair could be considered as an atypical toxin/anti-toxin system.

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RNase J of *Chlamydomonas reinhardtii*

Anna Liponska[1] , Soumaya Laalami[1] , Richard Kuras[2] , Loreto Suay[2] , Enrico Garbe[1] , Francis-André Wollman[2] and Harald Putzer[1]

[1] CNRS UMR8261 (Associated with Université Paris Diderot, Sorbonne Paris Cité), Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France
[2] CNRS UMR7141 (Associated with Université Pierre et Marie Curie), Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

Corresponding author: Harald Putzer: putzer@ibpc.fr

Chloroplasts are semi-autonomous organelles, derived from prokaryotic ancestors – cyanobacteria. Their gene expression relies partly on mechanisms derived from eucaryotes, as well as from prokaryotes. In the chloroplast, mRNA cleavages appear important for two processes : i) maturation of the transcript to protect it from nucleolytic attack and to allow its translation and ii) in mRNA degradation. The unicellular algae *Chlamydomonas reinhardtii* (Cr) has an RNase J ortholog encoded in the nucleus. The gene encodes a protein of 920 aminoacids, which is much longer than its bacterial counterparts, e.g., 555 aa for *B. subtilis* RNase J1. The *Chlamydomonas* enzyme contains a signal sequence that clearly suggests that this protein is addressed to the chloroplast.

We have cloned, expressed and purified CrRNase J and analyzed its activity *in vitro* for the presence of endonucleolytic and 5'-3' exonucleolytic activities on the *B. subtilis thrS* leader transcript. This substrate is routinely used to test these two activities of BsRNase J1. We also used 5' end labelled monophosphorylated oligonucleotides, differing in their first nucleotide (A,C,G or U), to verify if the first nucleotide can influence the 5'-3' exonucleolytic activity of CrRNase J. CrRNase J protein showed high endo- but no significant 5'-3' exonucleolytic activity. Moreover, we performed a yeast double-hybrid (Y2H) screen to look for potential partners of the CrRNase J that could enhance its 5'exo activity. We tested some of them (FTT2, NDA3, EF3 or HP4), in the *in vitro* test with CrRNase J.

We also tested CrRNase J *in vivo* in a heterologous system (*B. subtilis*). We inserted CrRNase J in a single copy on the *B. subtilis* chromosome in strains deleted for BsRNase J1, (delta *rnjA*) and/or BsRNase J2 (delta *rnjB* single and double mutations). The expression of CrRNase J had no effect on growth of a wild type strain, but could not compensate for the severe growth defect caused by the lack of BsRNase J1. Further analysis of two well-studied processing/degradation events that require the 5'-3' exonucleolytic activity of the *B. subtilis* enzyme (16S rRNA and *glmS*) in these recombinant strains proved that this enzyme was unable to provide the 5' exonuclease activity required for these processes in *B. subtilis*.

To our surprise, we found that Cr RNase J has no significant intrinsic 5' exonuclease activity that would justify its implication in the 5' maturation processes in *Chlamydomonas*. We conclude that either Cr RNase J is not the major 5' exonuclease that it was thought to be or it requires co-factors to induce its latent activity. Despite the fact that we did not succeed in identifying this potential co-factor we still favor, and cannot exclude, the hypothesis that a yet unknown chloroplast protein might activate and thus control CrRNase J as an exoribonuclease.

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Lauréats Prix Nine Choucroun

- **Stanislas VON EUW**

Bone biomineralization: from the structural characterization of the mineral to its 3D organization.

- **Joël LEMIÈRE**

Actin cytoskeleton and membrane shaping: from liposome to cellular reconstitution.

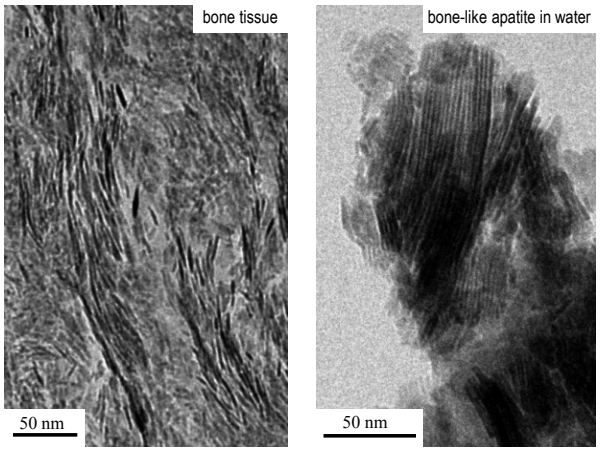
Bone biomineralization: from the structural characterization of the mineral to its 3D organization

Stanislas Von Euw, Thierry Azaïs*, Nadine Nassif*, Yan Wang & Florence Babonneau

Laboratoire Chimie de la Matière Condensée de Paris, UMR 7574 CNRS, UPMC UnivParis06,
Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France
*thierry.azais@upmc.fr *nadine.nassif@upmc.fr

This project aims to understand the mechanisms that allow the bone tissue to precipitate, and subsequently to organize a mineral matrix. This biologically controlled precipitation, called biomineralization, is poorly understood because it results from highly complex, dynamic, and coordinated biophysical and biochemical process. These processes lead to the formation of an endoskeleton in which the biologically formed mineral is calcium phosphate in the form of apatite.

Our approach to shed light on these complex processes was the deep characterization of the chemical structure, the chemical composition, as well as the physicochemical properties of bone apatite. The originality of this work was to study a fresh bone sample, analyzed within two hours after its extraction from the animal (*i.e.* a two years old sheep). This approach avoids any alteration of the bone mineral, and the later conserves its natural state of hydration. Such experimental rigor led to the evidence that the bone apatite particles have strong hydrophilic properties[1]. Furthermore, it was evidenced that synthetic bone-like apatites show analogous hydrophilic properties once studied in artificial wet conditions. It was found that the origin of these hydrophilic properties is related to the presence of a particular mineral domain located at the surface of the bone and bone-like apatite particles: the so-called non-apatitic domain[1]. It was highlighted that this non-apatitic domain is close to amorphous calcium phosphate in terms of chemical structure, chemical composition, and hydrophilic properties[1]. Regarding the chemical composition, a list of the ionic species that compose the bone apatite has been made with the help of original solid-state NMR techniques[1] – and it has been shown that the non-apatitic domain is mainly composed of divalent species: Ca^{2+}, CO_3^{2-} and HPO_4^{2-}. In addition, X-ray diffraction and cryo-electron transmission microscopy experiments were carried out to study the behavior of various apatite samples in aqueous media. Surprisingly, it was evidenced that the water molecules strongly adsorbed onto the non-apatitic domain can promote the 3D organization of the bone mineral *in vivo*[1].



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Actin cytoskeleton and membrane shaping: from liposome to cellular reconstitution

Joël Lemière[1,2,3]*, Valentina Caorsi[3], Cécile Sykes[3]

[(1)]Department of Molecular Biophysics and Biochemistry, Nanobiology Institute, Yale University, New Haven, CT, USA.
[(2)]Univ. Paris Diderot, Sorbonne Paris Cité, F-75205, Paris, France.
[(3)]Institut Curie — Centre de Recherche, Biomimetism of Cell Movement group, CNRS UMR 168, Physico-Chimie Curie, Université Pierre et Marie Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France.
* joel.lemiere@yale.edu

Cells move and change shape by dynamically reorganizing their cytoskeleton next to the plasma membrane. The biopolymer actin is a major component of the cytoskeleton that constantly assembles and disassembles, thus generating forces and stresses that deform the cell membrane. I address the role of actin and acto-myosin in organising membranes and controlling their shape. Based on former studies in the group of Cécile Sykes I developed new systems of actin shells formed at beads surface or at the cell-sized liposome membrane through actin polymerization and used skeletal myosin motors to induce shell contraction [(1,2).] This system mimics the function of two different actin structures. One is the lamellipodium at the cell front, where actin polymerization is activated at the membrane and pushes the membrane forward. The other one is the cell cortex, a sub-micrometer thick actin shell right beneath the cell membrane, which contributes to cell tension with the action of molecular motors. I showed that cortical tension around a cell-sized liposome can be generated by distinct mechanisms, all able to induce a spontaneous polarization of actin networks via symmetry breaking [(3)]. Finally, Cell-sized liposome doublets covered with either a stabilized actin cortex, or a dynamic branched actin network polymerizing at the membrane were created to dissect the individual contributions of actin polymerization and myosin to tension increase with a non-invasive method [(4)].

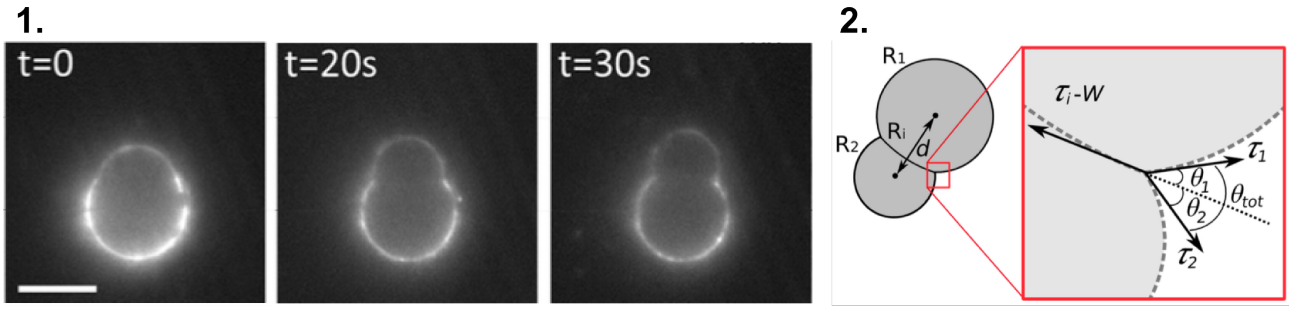

Figure. 1: Preformed actin filaments marked with Alexa 488 in the presence of myosin are photo-damaged by exposure to light. Photo-damaging generates tension release as observed through the decrease of the total contact angle. 2: Schematic of the doublet with the three characteristic radii. Inset: enlargement of the contact interface between the two liposomes with the Young's tension vectors and the contact angles.

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Conférence Edmond de Rothschild

Professeur Mei HONG du département de Chimie du MIT

Structure, Dynamics and Mechanism of Action of the Influenza M2 Protein from Solid-State NMR Spectroscopy

Structure, Dynamics and Mechanism of Action of the Influenza M2 Protein from Solid-State NMR Spectroscopy

Mei Hong*, Sarah Cady, Fanghao Hu, Wenbin Luo, Shu Liao, Jonathan Williams, Tuo Wang, Byungsu Kwon, Keith Fritzsching, Yongchao Su

Massachusetts Institute of Technology
* meihong@mit.edu

The influenza virus M2 protein forms an acid-activated tetrameric proton channel that is important for the virus lifecycle. The proton channel activity of influenza A virus M2 (AM2) is inhibited by the adamantane family of antiviral drugs. M2 also mediates membrane scission during virus budding. Solid-state NMR spectroscopy has provided rich structural information about the M2 protein bound to phospholipid bilayers that mimic the eukaryotic membranes of cells and virus envelopes [1]. Using solid-state NMR, we have 1) constrained the conformation, orientation and tetrameric assembly of the four-helix bundle formed by the transmembrane domain of M2 [2], 2) identified the pharmacologically relevant drug binding site of the protein and the orientation and dynamics of the bound drug [3], 3) revealed the sidechain conformation and motion of the proton-selective residue, a histidine [4], 4) determined the rates and equilibrium constants of proton exchange between water and histidine [5] and the channel hydration at different pH, 5) delineated the sidechain conformation and dynamics of the gating residue, a tryptophan [6], 6) identified the pH-dependent multiple conformational states of the proton-selective histidine and the modulation of this structure distribution by the extra-membrane domains of M2 [7]. These results indicate that histidine shuttles protons from water molecules into the virion, and uses ring motion and tautomerization to regenerate the initial conformation for the next proton relay. Tryptophan regulates the proton flux by periodic cation-π interactions with histidine at low pH. Amantadine inhibits this proton conduction process by blocking the N-terminal pore and dehydrating the channel. For the membrane-scission and virus-budding function, we have investigated how an amphipathic helix in the protein causes high membrane curvature [8]. Finally, the M2 protein of the influenza B virus (BM2) has little sequence similarity with AM2 except for the two functional residues, histidine and tryptophan. BM2 is also not yet druggable. Our latest structural data of membrane-bound BM2 reveal differences in the channel hydration and water-histidine proton exchange dynamics from those of AM2, giving insight into how nature has designed a functional analog with different amino acid residues. A striking feature of the M2 protein revealed by these spectroscopic data is that the protein possesses substantial structurally plasticity, which allows it to respond to changing environmental conditions such as pH and membrane composition to tailor its structure and dynamics to carry out its multiple functions. This work demonstrates the versatile ability of solid-state NMR spectroscopy to provide atomic details of the structure and dynamics of membrane proteins in lipid bilayers.

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