POSTERS ABSTRACTS
(Including short talks and workshops)

**Functional analysis of postbiotics underlies symbiotic relationships between the gut microbiome and the host genome**

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**Background.** The gut microbiota is a complex system of mutualistic microorganisms, hosting an impressive 100 trillion bacteria representing 1-3% of body mass and encoding for over 4M genes. It sits at the interface between the environment and the host organism and contributes to nutritional processes through many bacterial-specific metabolic reactions.
**Aim.** Our objectives were to identify biological relationships between the gut microbiome and the host genome.
**Methods.** We used mass spectrometry (MS)-based metabolomic profiling to acquire quantitative data for metabolites present in human serum samples that were tested for correlations with clinical and biochemical data in a population of 138 patients with coronary artery disease and controls.
**Results.** Among the 14,879 spectral data acquired by MS, statistical analysis of association to disease phenotypes primarily focused on 101 known metabolites. A total of 39 metabolites were associated with at least one clinical or biochemical phenotype, including several products of gut microbial metabolism. Among these, the microbial metabolite 4-cresol, which derives from the bacterial metabolism of tyrosine, was negatively correlated with obesity and diabetes. To explore the *in vivo* impact of 4-cresol on cardiometabolic phenotypes, we developed a phenotyping pipeline based on chronic subcutaneous delivery of 4-cresol by osmotic pumps in rodent models fed chow diet or obesogenic high fat diet. 4-cresol treatment resulted in reduced body weight, adiposity and adipocyte size, enhanced glucose-stimulated insulin secretion and pancreas weight, and increased pancreatic islet density, insulin positive area and β-cell proliferation. Molecular experiments demonstrated that DYRK1A, a known inhibitor of β-cell proliferation in humans, is a cellular mediator of 4-cresol. Further analyses of the full metabolomic dataset identified clusters of co-regulated metabolic signals providing a structural organization of human metabolism.
**Conclusion.** Our results illustrate the power of metabolomics to identify metabotypic endophenotypes underlying both disease risk and functional readouts of the gut microbiota.

**Targeting of a model amphipathic helix to lipid droplets**

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Lipid droplets (LDs) are dynamic organelles that play an essential role in cellular lipid homeostasis and are implicated in many human pathologies (obesity, diabetes, cancer, etc.); however, the mechanisms of selective protein targeting to LDs to mediate their function are poorly understood. Many LD proteins interact with LDs via amphipathic helices (AHs), which can mediate direct and reversible binding to lipid surfaces, and are also present in numerous non-LD proteins. We use a uniquely long and monotonous AH, found in the mammalian LD protein perilipin 4, to probe the physico-chemical properties of the LD surface. We show that this AH is unstructured in solution but can adopt a highly helical conformation, over a length of hundreds of amino acids, when in contact with a lipid surface. The regularity of its amino acid sequence allows us to introduce subtle mutations that are repeated along the length of the helix in order to dissect the parameters that are important for AH targeting to cellular LDs. By this mutagenic approach, we show that LDs are relatively permissive for AH binding, suggesting a surface with abundant lipid packing defects, in agreement with predicted behavior of a phospholipid monolayer on a neutral lipid core (*Bacle et al., 2017, Biophys J, 112:1417*). We show that AH length, hydrophobicity and charge all contribute to LD binding. However, a small increase in the hydrophobicity of AHs that leads to improved LD localization also makes them more promiscuous for binding to other cellular compartments. These results suggest that the physico-chemistry of the perilipin 4 AH is exquisitely tuned to be specific for LDs. *In vitro*, we find that purified wild-type AH binds poorly to bilayer membranes. In contrast, it can interact efficiently with neutral lipids and is capable of forming small uniformly coated oil droplets. Accordingly, overexpression of this AH in cells overcomes a decrease in LD stability associated with phospholipid depletion. We propose that by substituting for the phospholipid monolayer, perilipin 4 may be important for stabilization of LDs when phospholipids are limiting, for example during periods of LD growth.

## Title

DNA-PK impedes DNA damage-induced transcription of stress response genes

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## Abstract

Organisms are continuously exposed to sublethal stresses, but how they respond to such stresses at the molecular level is still largely unknown. One known mechanism is genetic regulation of stress response proteins, such as HSP72 - which is encoded by both HSPA1A and HSPA1B genes. We previously reported that HIRA, a histone H3 chaperone, is prompted by sublethal stresses to establish acquired tolerance to subsequent lethal stresses in both *S. pombe* and human fibroblasts. This suggested that chromatin remodeling plays a role in sublethal stress response. Here, we determined that DNA damage response (DDR) factors and topoisomerases are potent histone H3 interactors and identifed their role in the regulation of stress response in human fibroblasts. Our results indicate that sublethal stress induces DNA double-stranded breaks (DSBs) by topoisomerase IIA (TOP2A). Moreover, we show that following sublethal heat stress, histone variant H3.3 and its interactors, including DDR factors and TOP2A, are enriched at the transcription start site and/or open reading frame of the HSPA1A/B loci. Lastly, we determined that DDR protein DNA-PK is a negative regulator of HSPA1A/B expression following DSB-induced transcription. Based on our results we propose that in response to sublethal stresses, HIRA/H3.3 and DNA-PK counter-regulate stress response genes to balance transcription and DNA damage response, for immediate survival and genomic stability, respectively.

# Development of an AI-assisted algorithm for the prediction of novel causal genes and variants for mendelian disorders from whole genome sequencing

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Advances in DNA sequencing technologies have now enabled the rapid and cost-efficient identification of casual genes and variants for a number of diseases. This is especially true for Mendelian disorders, where patients who carry a causative variant in their genome, can finally obtain a definitive diagnosis on their disease. However, even with this revolutionary technology, the actual success rate of genetic diagnosis via next-generation sequencing is currently only at around 30% for undiagnosed Mendelian disease cases. This is in part due to the limitations of the analytical methods that are available to identify and prioritize casual variants from the vast amounts of sequencing data generated.

Currently, the genetic diagnosis of Mendelian disorders is performed by comparing the genome of a patient to those of a large number of controls. Such comparisons generally produce a large list of genetic variants that are unique to the patient. Many of these are probably benign and identifying the causal gene and variant can be a real challenge. To address this problem, we have developed a novel method that ranks candidate genes and variants using an AI-assisted algorithm that relies on IBM Watson's text mining approach. As a proof of concept, we used a large whole-genome sequencing (WGS) dataset on Retinitis pigmentosa (RP) with 523 cases and 2,143 controls. Our method consists of the following steps:

1) Select the inclusion criteria of variants to maximize the difference between true positive rate for patients and false positive rate for controls based on previously known causal genes from a public database.
2) Using this inclusion criteria, create a list of candidate genes and variants.
3) Use IBM Watson to sort and prioritize this list of genes.

Using this strategy on the RP WGS dataset, we were able to identify and priority 994 candidate genes. Notably, many of our top ranked genes shared structural and functional features with previously known RP genes. We also succeeded in increasing the diagnosis rate of RP from 37% to 52% by incorporating these top ranked candidates without increasing the rate of false positives in controls.

Going forward we plan to further improve the approach by integrating other AI technologies that rely on omics or image analysis data. We also plan to develop a gene and variant registry with the aim of constructing a comprehensive infrastructure in Japan for studying the genetics of intractable diseases. In this registry, various AI technologies will be implemented to perform integrative analyses across various diseases.

# Recombination of DENV 2 NS5 C-Terminal 70 kDa Fragment and Its Potential Application

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Dengue virus (DENV) infection in humans may cause self-limited Dengue fever (DF), or even clinically more severe Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). It is a mosquito-borne infectious disease mainly in tropical and subtropical regions, and is becoming one of the fastest growing infectious diseases in recent years. People are relatively sensitive to first infection with whichever of the four serotypes and obtain the lasting immunity to the same serotype virus. But, secondary infection of different serotype virus is more likely to cause DHF/DSS because of the antibody dependent enhancement (ADE), leading to high morbidity and mortality. So far, there is no antiviral drug against DENV, thus rapid diagnosis and symptomatic treatment is particularly important.

Non-structural protein 5 (NS5) of DENV is the longest and the most conserved protein amongst the *Flaviviridae* family, which plays a vital role in viral life cycle through replication of viral RNA, mostly because of its C-terminal 70 kDa fragment (NS5/C70) predicted as RNA dependent RNA polymerase (RdRp) structure.

In this study, DNA fragment of the NS5 RdRp domain was synthesized according to GenBank accession number AF038403.1, and the expression plasmid pQE30-NS5/C70 was constructed and transformed into *E coli* M15. Then we obtained soluble expression of the recombinant polypeptide segment of NS5/C70 after optimizing the expression conditions.Further, the purified recombinant NS5/C70 was coated and reacted to serums of the four serotype patients, with a group of uninfected normal serums used as negative control. The ELISA results showed that the recombinant NS5/C70 not only positively reactivity to DENV-2 infected serum, but also positive to the other three serotype serums.

We assume the recombinant polypeptide may be used for primary or rapid diagnosis of DENV infection, saving the time coating all the four types of antigens. Meanwhile, based on its non-different serotype reactivity, it might be used for vaccine development without causing ADE which.

# Effect of multi-drug resistance ABC transporter activity on their conformation-induced redistribution on membranes

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Integral trans-membrane proteins are involved in various cellular functions and their dysfunction is associated with human pathologies [1]. The lipid-protein interactions have been studied to address structure-function relationship of transmembrane proteins at molecular level. However, the effects related to membranes physical properties on trans-membrane proteins have not been well-studied, and not at all when their conformations change. Recent experimental evidence indicates the intrinsic interplay between protein shape and the properties of its membrane environment [2,3]. It is expected that non-cylindrical proteins tend to cluster and be enriched in curved membranes. Thus, we studied BmrA a bacterial ATP binding cassette (ABC) transporter from *B. subtilis* involved in export of a large diversity of substrates in an ATP dependent manner, fairly homologous to human P-glycoprotein [4]. The conformational change in nucleotide-binding domains (NBDs) of BmrA between apo and the post-hydrolytic state (tweezers-like motion) is 5 nm and that is the largest tweezers motion reported till date in the case of trans-membrane proteins. Here we addressed how the conformational dynamics of BmrA influence its membrane properties, in particular its spatial distribution on flat or curved membranes.

To decipher the effect of the conformational dynamics of BmrA on its spatial distribution in membranes, depending on membrane curvature, we used cell-sized giant unilamellar vesicles (GUVs) containing either the apo- or closed-conformation BmrA to form membrane nanotubes with controlled radii. We found that, at low protein density, apo-BmrA is highly enriched (50 times) in nanotubes as compared to flat membrane and simultaneously modulates tube radius from 100 nm to 30 nm, due to its high intrinsic curvature. Surprisingly, although the post-hydrolytic closed-conformation BmrA is expected to be cylindrical, we measured an enrichment of this conformation in nanotubes, but about 3 time less pronounced then for apo-BmrA. Eventually, in the presence of ATP, BmrA has reduced curvature selectivity as compared to the apo form, in agreement with a cycling change of conformation between the apo and the closed forms. This study on reconstituted transmembrane proteins demonstrates that protein distribution on membranes is influenced by the interplay of membrane curvature, effective shape and flexibility of membrane proteins.

[1] Gottesman M. et al., (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2: 48-58
[2] Quemeneur F. et al., (2014) Shape matters in protein mobility within membranes. PNAS 111:5083-5087
[3] Aimon S. et al., (2014) Membrane shape modulates trans-membrane protein distribution. Dev Cell 28: 212-218.
[4] Lubelski J.et al., (2007) Distribution and Physiology of ABC-Type Transporters Contributing to Multidrug Resistance in Bacteria. Microbiol Mol Biol Rev 71: 463-476

# Membrane reshaping by curvature sensitive septin filaments

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Septins are cytoskeletal proteins able to form supramolecular structures such as filaments, networks and rings. They are bound to the inner plasma membrane at specific location including the separation between the mother and daughter cell during cytokinesis and the basis of cilial cells. Septins are essential for cytokinesis, participate in the formation of diffusion barrier and might be involved in membrane deformation and rigidity.

Using simplified biomimetic systems, we asked if septins were sensible to curvature, given their preference to be located at places of high curvature. To mimic specific curvatures and geometries observed in vivo, we have used PDMS substrates covered with a supported lipid bilayer. “Wavy” PDMS patterned substrates display both positive (“bumps”) and negative (“valleys”) curvatures. To our surprise, we have seen, using Scanning electron Microscopy, that Septin filaments have a preference for negative micrometric size curvatures. On positively curved geometries (bumps), septins spontaneously align along the “bumps” and thus orient towards null curvature.
This curvature preference is closely related to the ability of septins to reshape and deform membranes. When interacting with giant unilamellar vesicles, septins induce µm scale deformations with the formation of regular rigid spikes at the surface of the liposomes.
We propose a theoretical model to take into account these observations and could be relevant to describe the organization of septins during cytokinesis in vivo.

# ROMA: Representation and Quantification of Module Activity from Target Expression Data

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## Abstract

In many analysis of high-throughput data in systems biology, there is a need to quantify the activity of a set of genes in individual samples. In cancer the same pathway can be affected by defects in different individual genes in different patients and application of gene set approaches in the analysis of genomic data can help to capture biological information that is otherwise undetectable by focusing on individual genes.

We present here ROMA (Representation and quantification Of Module Activities) software, designed for fast and robust computation of the activity of gene sets (or modules) with coordinated expression. ROMA activity quantification is based on the simplest uni-factor linear model of gene regulation that approximates the expression data of the gene set by its first principal component.

The proposed algorithm implements novel functionalities: it allows to identify which genes contribute mainly to the activity of the module; it provides several alternative methods for principal components computation, including weighted and centered versions of principal component analysis; it distinguishes overdispersed modules (based on the variance explained by the firts principal component) and coordinated modules (based on the significance of the spectral gap); finally, it computes statistical significance of the estimated module overdispersion. ROMA can be applied in many contexts, from estimating differential activities of transcriptional factors to finding overdispersed pathways in single-cell transcriptomics data. We present here the principles of ROMA providing a practical example of its use. We applied it to compare distinct subtypes of medulloblastoma disease in terms of activated/inactivated signalling pathways and transcritpional programs.

[1] Martignetti L, Calzone L, Bonnet E, Barillot E, Zinovyev A. ROMA: Representation and Quantification of Module Activity from Target Expression Data. Front Genet. 2016. 7:18.

**EPIGENETICS : OPEN QUESTIONS**

*In this workshop, I will review the molecular bases of Epigenetics, as well as some essential but yet unsolved questions, which will be a subject of further discussion. As a part of it, a new hypothesis of the role of small non-coding RNAs in epigenetics will be presented*.

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2.Nadya Morozova (CNRS/I2BC,IHES )

Geometry of Morphogenesis

The process of morphogenesis is an evolution of a shape of an organism together with the differentiation of its parts.

It is clear that differential gene expression, being a necessary part of the process of pattern formation, cannot explain the formation of a precise geometry of an organism and its parts in space.

It looks plausible to suggest the existence of an additional biological code (epigenetic code) which bears information about geometrical pattern of an organism and thus coordinates the cascades of molecular events implementing a pattern formation (e.g. differential gene expression, directed protein traffic, growth of cytoskeleton).

Here we suggest a set of postulates underlying a pattern formation process together with its possible mathematical formalization, which allows discovering a nature of such a code and a correspondence between this code and its realization in a particular determined geometry in space and time.

**Extracting individual variability in the immune response to seasonal influenza vaccination**

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The human immune system is known to be highly variable among individuals, but it is not well understood how the variability changes over time, especially when faced with external perturbations. Here we analyzed individual variability in the immune system in a cohort of 301 Japanese volunteers who received the same trivalent inactivated influenza vaccine in winter 2011. To extract important variability axes from single-cell measurements in a data-driven and unsupervised manner, we devised a computational method termed LAVENDER (latent axes visualization and evaluation by nonparametric density estimation and multidimensional scaling reconstruction). It measures distances between samples using *k*-nearest neighbor density estimation and Jensen-Shannon divergence, then reconstructs samples in a new coordinate space, whose axes can be compared with other omics measurements to find biological information. Application of LAVENDER to multidimensional flow cytometry datasets of B and T lymphocytes (taken before and 1, 7, 90 days after vaccination) uncovered an axis related to time and another axis related to individuality. We found that the values of the individuality axis were positively correlated between different days, suggesting that the axis reflects the baseline immunological characteristics of each individual. In fact, the value of the axis before vaccination was highly correlated with the neutrophil-to-lymphocyte ratio, a clinical marker of the systemic inflammatory response; this was verified by the transcriptome analysis of peripheral blood. These results demonstrate that LAVENDER is a useful tool for identifying critical heterogeneity among similar but different single-cell datasets.

# The dynamics of gene expression in vertebrate embryogenesis at single cell resolution

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## Abstract

Time series of single cell transcriptome measurements can help us reconstruct the dynamics of cell differentiation in both embryonic and adult tissues. We produced such a time series of single cell transcriptomes from whole frog embryos, spanning zygotic genome activation through early organogenesis. From the data we derive a detailed catalog of cell states in vertebrate development, and show that these states can be assembled into temporal maps tracking cells as they differentiate over time. The inferred developmental transitions recapitulate known lineage relationships, and associate new regulators and marker genes with each lineage. We find that many embryonic cell states appear far earlier than previously appreciated, and assess conflicting models of vertebrate neural crest development. By further incorporating a matched time series of zebrafish from a companion paper, we perform global analyses across lineages, time, and species, revealing similarities and differences in developmental gene expression programs between frog and fish.

# CAMP AND MEMBRANES COOPERATE TO ACTIVATE THE RAPGEF EPAC1 IN HEART PHYSIOLOGY

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The guanine nucleotide exchange factor (GEF) Epac1 activates the small GTPase Rap1 in cardiovascular physiology by stimulating exchange of GDP for GTP. It is currently considered a potential therapeutic target, which is motivating intense research in drug discovery. EPAC1 has a complex regulatory regime, in which binding of the second messenger cyclic AMP (cAMP) promotes a large conformational change leading to auto-inhibition release. In this study, we investigated the role of membranes in EPAC1 activity by reconstitution of EPAC1 and Rap1 in artificial membranes combined with fluorescence-based kinetics, structural analysis and inhibition by a small molecule. We found that cAMP and membranes cooperate to potentiate EPAC1 activity by more that 2 orders of magnitude. We identified structural determinants for this effect, and used a small molecule that blocks EPAC1 activity to highlight intermediates between the auto-inhibited and the fully active conformations. These findings demonstrate a major contribution of membranes to EPAC1 structure and activity, which can inspire new routes for the discovery of drugs against cardiovascular diseases.

# Network analysis of functional genomics screening data

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"Omics" and in particular functional genomics studies rely on high-throughput experimental approaches aiming at identification of a set of biological components (genes, mRNA, miRNA, proteins) relevant to a given phenotype. Such studies commonly generate large datasets, usually presented in the form of a ranked list of biological components, from which a so-called 'hit list' can be retrieved by applying an appropriate statistical threshold. While this is usually sufficient to identify key biological components relevant to a given phenotype, ranking methods fail to provide a broader picture of the biological system under study. Here is where network analysis comes in. We present a new exploratory network analysis method to uncover potential members of molecular pathways contributing to the phenotypic changes. The method works on an integrated network (with protein-protein, transcriptional, miRNA-mRNA, metabolic interactions), uses hit list dataset from "omics" study and employs the shortest path approach as well as centrality index.

# Junctional clustering of ADAM10 by the PLEKHA7-PDZD11 complex through Tetraspanin33 promotes cell death by *Staphylococcus aureus* α-toxin.

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## Abstract

Although transmembrane proteins of cell-cell junctions can function as receptors for viruses and bacterial toxins, the role of cytoplasmic junctional proteins in host-pathogen interactions is poorly understood. The cytoplasmic junctional protein PLEKHA7 was identified as a key host factor for cell death caused by *Staphylococcus aureus* α-toxin, through a screen in Hap1 cells. Here we clarify the mechanism through which PLEKHA7 promotes cell death. Using immunofluorescence analysis, we demonstrate that PLEKHA7 and its interacting partner PDZD11 cluster the α-toxin receptor ADAM10 and its partner Tspan33, localizing toxin pores at the *zonula ahaerens* of epithelial kidney cells and regions of cell contact of Hap1 cells. Toxin pores clustered at junctions are more stable, and cause cell death at low/intermediate toxin doses, suggesting that they have intrinsically enhanced cytotoxic activity. Proximity ligation and co-immunoprecipitation assays show that PLEKHA7 associates with the ADAM10-Tspan33 complex in a PDZD11-dependent manner, and GST pulldowns show that PDZD11 promotes the interaction of N-terminal fragments of PLEKHA7 with the C-terminal cytoplasmic domains of Tspan33 and ADAM10. In summary, the PLEKHA7/PDZD11 complex controls the function of a pore-forming toxin by acting as a scaffold that organizes the surface distribution of its receptor complex. These results provide a novel molecular mechanism underlying host-pathogen interactions, and a new function for cytoplasmic components of adherens junctions.

# A multiscale model of early cell lineage specification including cell division

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Embryonic development is a self-organized process during which cells divide, interact, change fate according to a complex gene regulatory network and organize themselves in space. Here, we model this complex dynamic phenomenon in the context of the acquisition of epiblast (Epi) and primitive endoderm (PrE) identities from the inner cell mass (ICM) of the preimplantation embryo in mouse. The pluripotent Epi cell lineage is responsible for later development of the embryo, while PrE lineage gives rise to the extraembryonic tissue. Nanog and Gata6, two characteristic genes for Epi and PrE lineages respectively, are at first co-expressed in ICM cells. Later they become mutually exclusive which leads to the cell development of Epi and PrE cells. Through the extracellular communication, the two lineages stimulate their surrounding cells to adopt the opposite fate. Thus they form the so-called "salt-and-pepper" pattern, which is important for the balance of Epi and PrE cells in the embryo and the robustness of its early development. In this work, we have developed a 3D multi-scale model of early mammalian development [1], based on a previously proposed GRN exhibiting tristability [2,3]. The multiscale model describes cell division and interactions between cells, as well as biochemical reactions inside each individual cell and in the extracellular matrix. We use the model to study the Epi and PrE lineage development and the appearance of a so-called salt-and-pepper pattern which the two lineages form.

**Bragsin : a new interfacial protein/membrane inhibitor to block small GTPase signalling**

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Ras-like small GTPases and their regulators assemble signaling hubs at the surface of membranes to orchestrate numerous essential cell functions. As a consequence, their deregulation can lead to a myriad of pathologies such as cancers, cardiovascular diseases or infectious diseases, and drugs modulating their activity are highly sought-after. Despite their significance, small GTPase signaling nodes have remained elusive drug targets due to their structural flexibility, their flat topology lacking suitable cavities for small molecule inhibitors, extended protein-protein interfaces and multiple protein-lipid interactions that are poorly druggable by traditional competitive inhibitors.

The guanine nucleotide exchange factor (GEF) BRAG2/IQSec1 activates Arf small GTPases by promoting their GDP/GTP exchange at the membrane-cytosol interface. As many GEFs, BRAG2 establishes multiple interactions with the membrane that allow it to reach a remarkable efficiency (Aizel *PLoS Biol* 2013, Karandur *PNAS* 2017) . BRAG2 regulates cell surface levels of cadherin and integrin receptors and has been implicated in invasiveness and tumor metastasis in several cancers, notably in breast cancer through a direct interaction with the EGF receptor (Morishige et al. *Nat Cell Biol* 2008). BRAG2 is thus an attractive therapeutic target but no specific inhibitor has been reported to date.

Here, we describe how the small molecule Bragsin efficiently and selectively inhibits BRAG2-mediated activation of Arf in cells. Using recombinant purified proteins, we show that Bragsin inhibit BRAG2 nucleotide exchange activity on Arf, although solely when the system is reconstituted on artificial membranes. Our crystallographic structure of Bragsin bound to BRAG2 reveals that Bragsin binds to the interface between the protein and the membrane. We propose that Bragsin specifically inhibits BRAG2 by altering the geometry of the GEF/membrane interaction without disrupting them. Finally, we show that Bragsin kills cancer stem cells, paving the way towards the development of new drugs. Altogether, Bragsin represents a novel class of interfacial inhibitors targeting protein/membrane interactions. Our study highlights a promising strategy for GTPase signaling inhibition by small molecules, which are highly needed to decipher molecular mechanisms involved in both normal and disease-related signaling pathways.

# Recombinant DENV 2 NS5: An Effective Antigen for Diagnosis of Dengue Infection

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Dengue fever as one of the most important mosquito-borne disease worldwide which approximately half of the world's population is at risk presenting several degrees of symptoms, including life-threatening dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue virus (DENV) belongs to the *Flaviviridae* family, and are classified into four immunologically distinct serotypes, DENV 1 to 4. Although, there is no specific treatment, early diagnosis is important for proper medical care to minimize mortality, and for prompt initiation of public health control measures. There are some diagnostic tests targeting NS1 antigen in commercial, but these methods need all the four serotypes of NS1. NS1 and NS5 are translated and appear almost simultaneously.

Our work is mainly based on the fact that NS5 is the most conservative protein among all the flavivirus family, and its' RNA dependent RNA polymerase (RdRp) domain plays a vital role in viral RNA replication. It is a prospective biomarker for dengue infection. In this study, we constructed the DENV 2 NS5 full length and DENV2 NS5 C-terminus RdRp domain (NS5-C70) expression plasmids, a 104 kDa fragment of NS5, and a 70 kDa fragment of NS5-C70 were respectively expressed in *Escherichia coli*. These two purified recombinant products reacted with sera of patients infected with dengue virus in an enzyme-linked immunosorbent assay with significantly higher optical density values compared with the control sera. The recombinant DENV 2 NS5 showed strong reactivity for any of the four serotypes, while the NS5-C70 fragment showed strong reactivity for DENV 2 and 4. It is suggested DENV 2 NS5 can detect all the four serotypes patients, greatly reducing the cumbersome procedures with four viral types of antigens in early diagnostic process. The recombinant NS5 protein might be an effective antigen for dengue diagnosis, and NS5-C70 also can be used for auxiliary diagnosis.