PSB

THE PARTNERSHIP FOR STRUCTURAL BIOLOGY

Florent Bernaudat – scientific coordinator

The Partners

International Institutions:

- Institut Laue Langevin

- European Synchrotron Radiation Facility

- European Molecular Biology Laboratory

National Institution:

- Institut de Biologie Structurale: CEA, CNRS, Université Joseph Fourier

“A European Centre of Excellence”

The collaboration brings together the remarkable expertise and facilities available for structural biology on this unique international campus.

The PSB in 4 dates

- **November 2002:** The PSB was established by a Memorandum of Understanding by the EMBL, the ESRF, the ILL and the IBS

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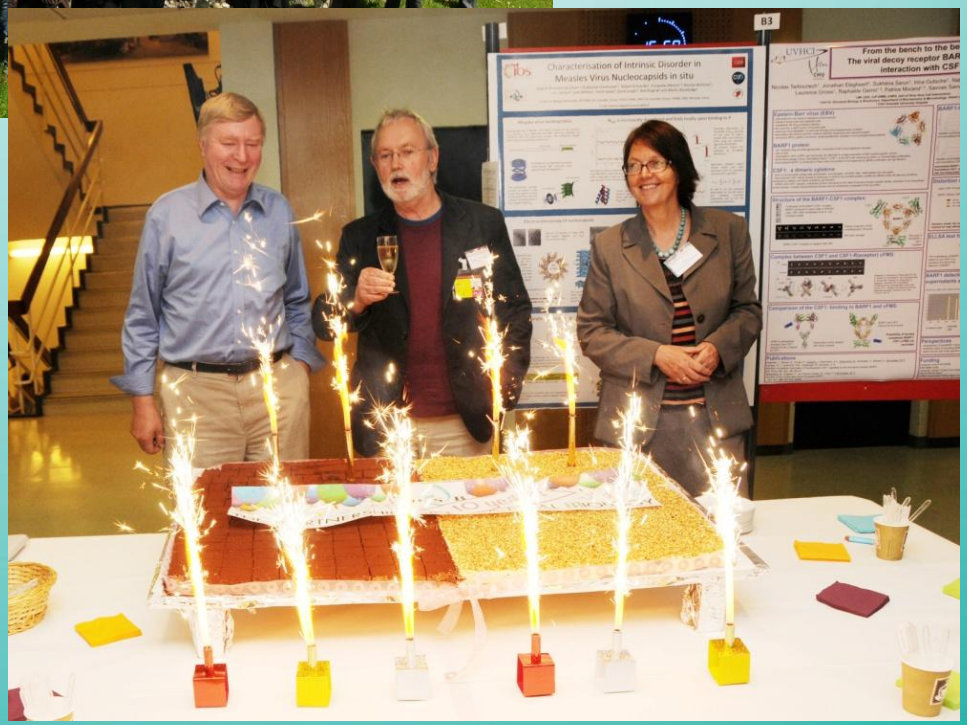
- **June 2013**: PSB 10th anniversary.

PSB 10th anniversary

June 4, 2013



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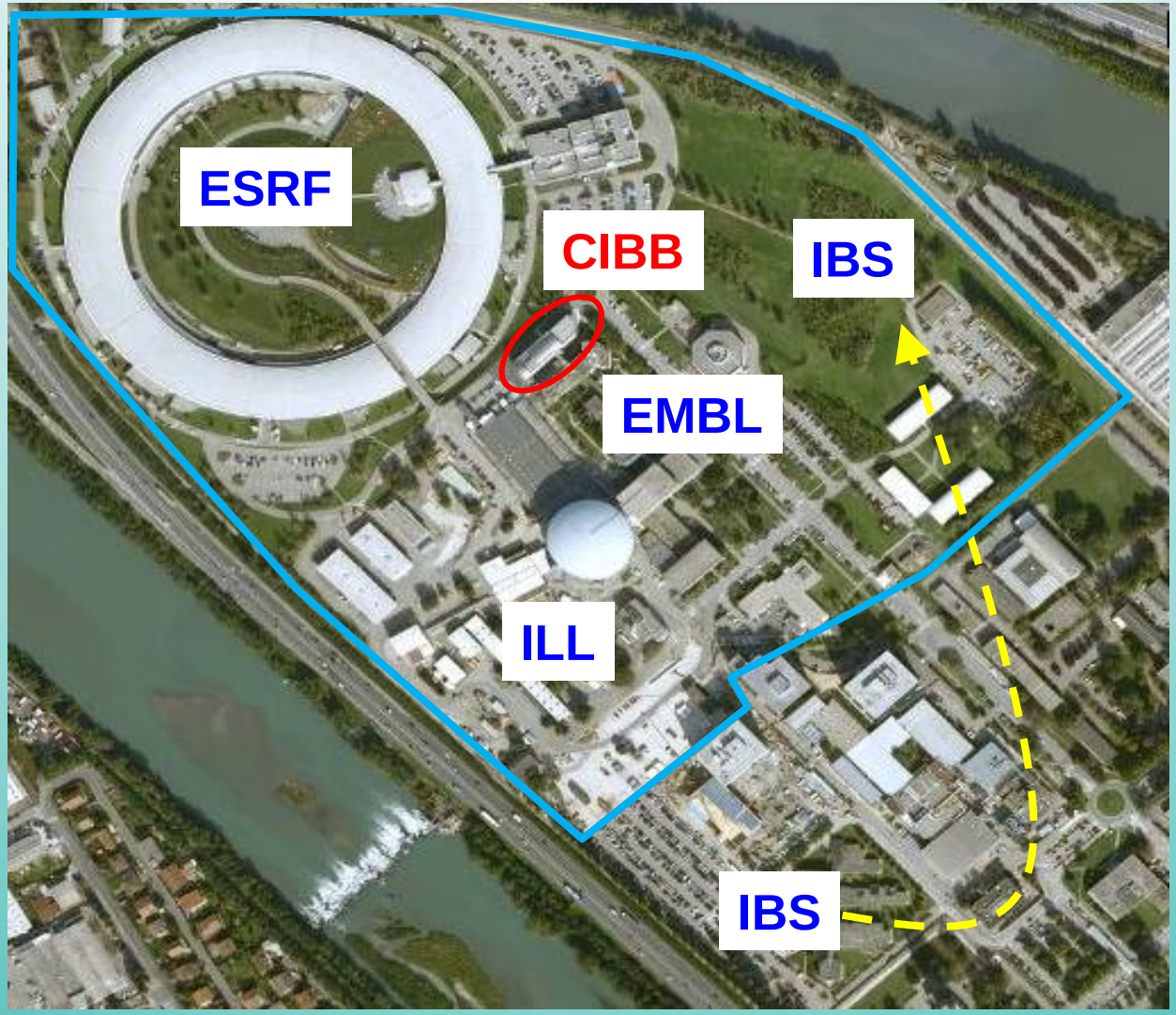
- **October 2013**: The IBS moved into the EPN campus



The Partnership for Structural Biology - Grenoble
350 scientists within walking distances

PSB Get Together

- meet your colleagues
- discuss your research
- interact
- collaborate
- participate

With posters from:

- HIV and Human Persistent Virus group - IBS (Pascal Poignard)
- Marcia group - EMBL (Marco Marcia)
- Life Sciences group - ILL (Trevor Forsyth)

ibs · NEUTRONS FOR SCIENCE · ESRF · EMBL

Cheese, wine and other refreshments

More information available on the PSB intranet website or psbgtog@gmail.com

PSB Student day

Next student day: Monday 7th March 2016

PSB Training activities

- Crystallography tutorials and SANS/SAXS courses for PSB Students
- EMBO Courses co-organised by the partners
- HERCULES
- Erasmus Mondus Program
- International Master in Structural Biology (Université Grenoble Alpes – Sep 2016)

PSB Science

broad and diverse

BIOLOGICAL RESEARCH

Host-Pathogen Interactions
- Bacterial pathogens
- Immunity
- Virology & viral infection

DNA/RNA & Gene Regulation
- Nucleic acid structure
- Gene regulation

Stress Responses in Prokaryotes
- Extremophilic bacteria
- Heavy atom homeostasis

Cell Division
- Eukaryotes
- Prokaryotes

Metalloproteins/Enzymology

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Metalloproteins/Enzymology

PSB: 200 peer-reviewed articles/year

15% multi-institute authorship

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TECHNOLOGY DEVELOPMENT

Methodologies for Structural Biology
Protein Expression
Crystallisation
Functional Studies
Structural Methods

Instrumentation
Synchrotron
Neutron scattering

PSB: a unique palette of **23** technological platforms for integrated structural biology studies

Protein Expression
Cell Free
ESPRIT
Eukaryotic Expression Facility
Deuteration Lab
Isotopic Labeling
Robiomol

Sample Characterization
Analytical Ultra Centrifugation
Biophysics
Cell imaging
Mass Spectrometry
Membrane Protein Purification Platform
NMR Quality Control
Protein Sequencing
Surface Plasmon Resonance

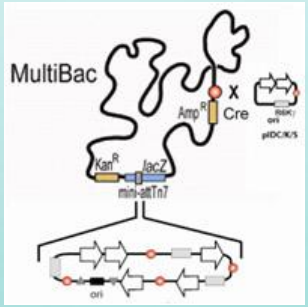
High Resolution Studies
Cryobench
FIP Beamline (BM30)
High Field Nuclear Magnetic Resonance
HT Crystallisation
HT Membrane Protein Crystallisation
Neutron Diffraction
Structural Biology Beamlines

Supramolecular Structures
Electron microscopy
SANS/ SAXS

Protein expression

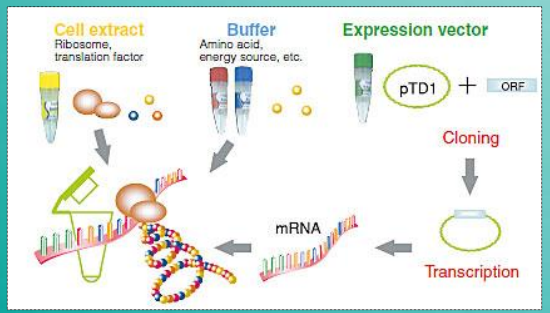
Robiomol

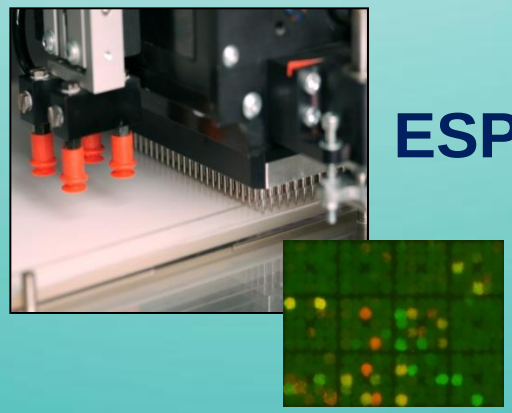
Eukaryotic Expression Facility



Isotopic Labelling

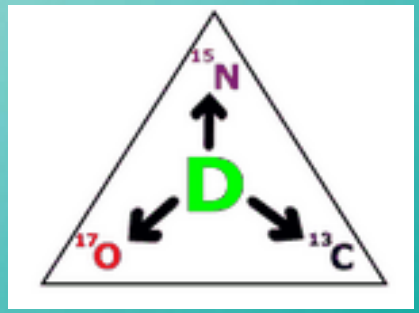
Cell Free





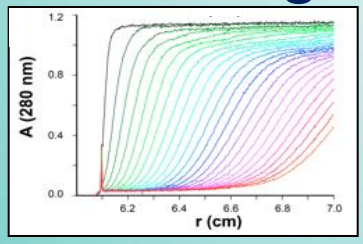
ESPRIT

Deuteration Lab



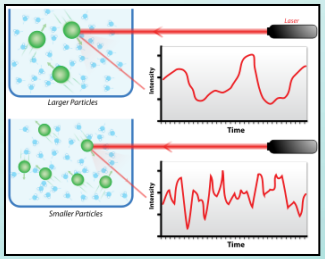
Sample Characterisation And Preparation

Analytical UltraCentrifugation

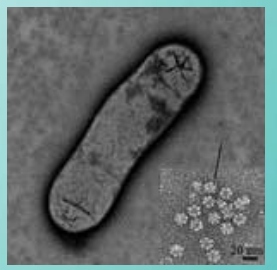


1-D NMR

Biophysics platform

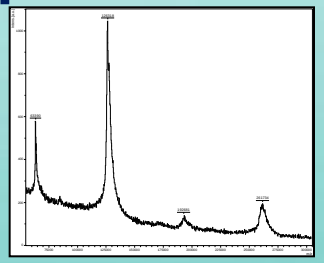


Negative stain EM

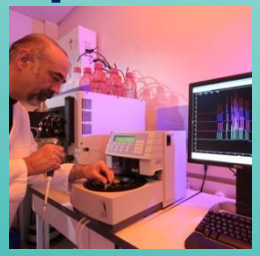


Multistep Protein Purification Platform

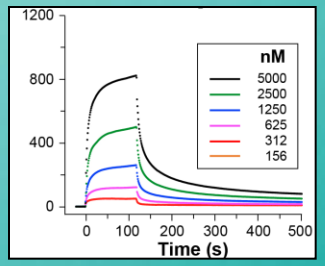
Mass Spectrometry



Protein Sequencing

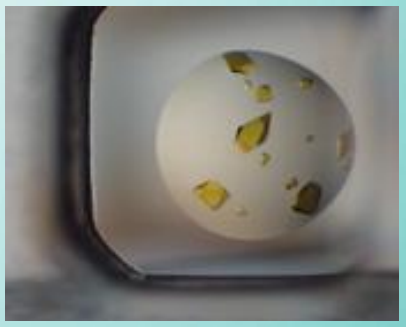


Surface Plasmon Resonance



Crystallisation

High Throughput Crystallisation (HTX)



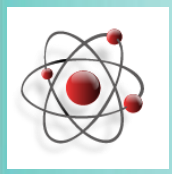
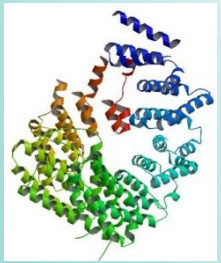
High Throughput Membrane Protein Crystallisation Platform (HTMPC)

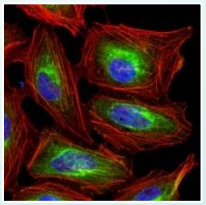
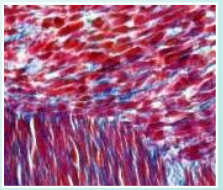
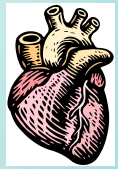
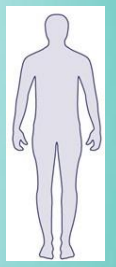
Crystallisation

Science and Innovation with Neutrons in Europe 2020

D-lab at ILL involved in large crystal growth project

Structure and dynamics

atoms proteins viruses bacteria cells tissues organs organisms

1 Å 1 nm 10 nm 100 nm 1 µm 10 µm 100 µm 1 mm 1 cm 1 m

SAXS/SANS

Cell imaging

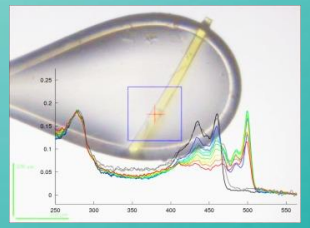
single-particle cryo EM

X-ray, neutron crystallography

High field NMR

Light microscopy

Cryobench spectroscopic studies



correlative microscopy

Cryo electron tomography

EM platform

120 kV , LaB6, NS
2.7k x 4k CCD

T12 (NS)

200 kV , FEG, Cryo
4k x 4k CCD

TF20 (cryo)

300 kV , FEG, Cryo, Tomo
4k x 4k K2 DDE 2k x 2k Gif, EPU

Polara (cryo)

Instruments

Vitrobot

Leica EM-UC7 + EM-FC7

Leica EM-IGL

High Field NMR Facility

- 6 NMR Spectrometers from 600 to 950 MHz
- State of the art detection probes
- ^{1}H, ^{2}H, ^{13}C, ^{15}N, ^{19}F & ^{31}P NMR
- Solution & Solid State NMR applications

Neutron diffraction beamlines

LADI-III: quasi-Laue neutron diffractometer

- Data collection at RT or cryogenic temperatures using Cobra cryostream;
  e.g. cryo-trapping studies of enzymatic reaction intermediates

- Optimized for high- to medium-resolution (1.5 – 2.5Å) studies of large (50 – 120Å on edge) unit-cell systems using perdeuterated crystals (0.05mm3 – 0.5mm3)

D19: thermal neutron monochromatic diffractometer

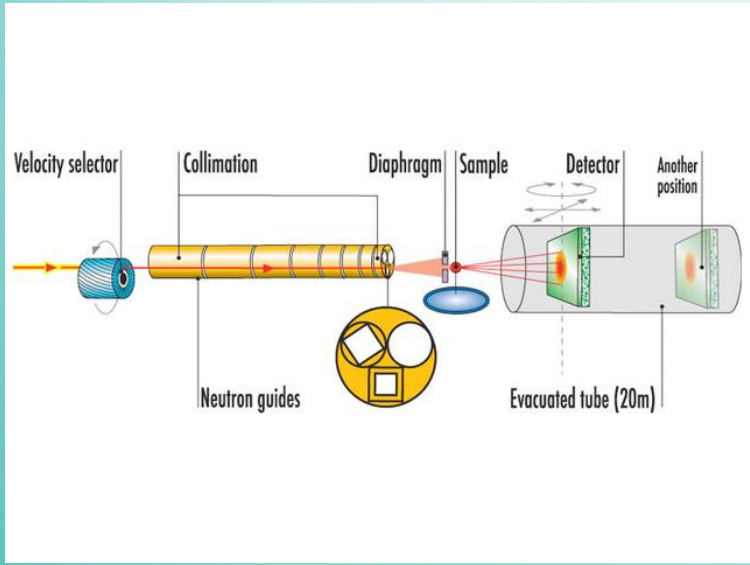
- Monochromatic data collection
  (λmono 0.8 – 2.4Å, e.g. λ = 1.46Å)

- Large (120° x 30°) PSD 'banana' detector

- Optimized for high-resolution (better than 1Å) studies of smaller (30 – 70Å on edge) unit-cell systems using crystals > 1mm3

SANS/SAXS platform

D22: small-angle neutron scattering diffractometer



BM29: BioSAXS

New mode of operation in 2016 for integrated access

How to access PSB platforms?

- Peer Review

  next deadlines

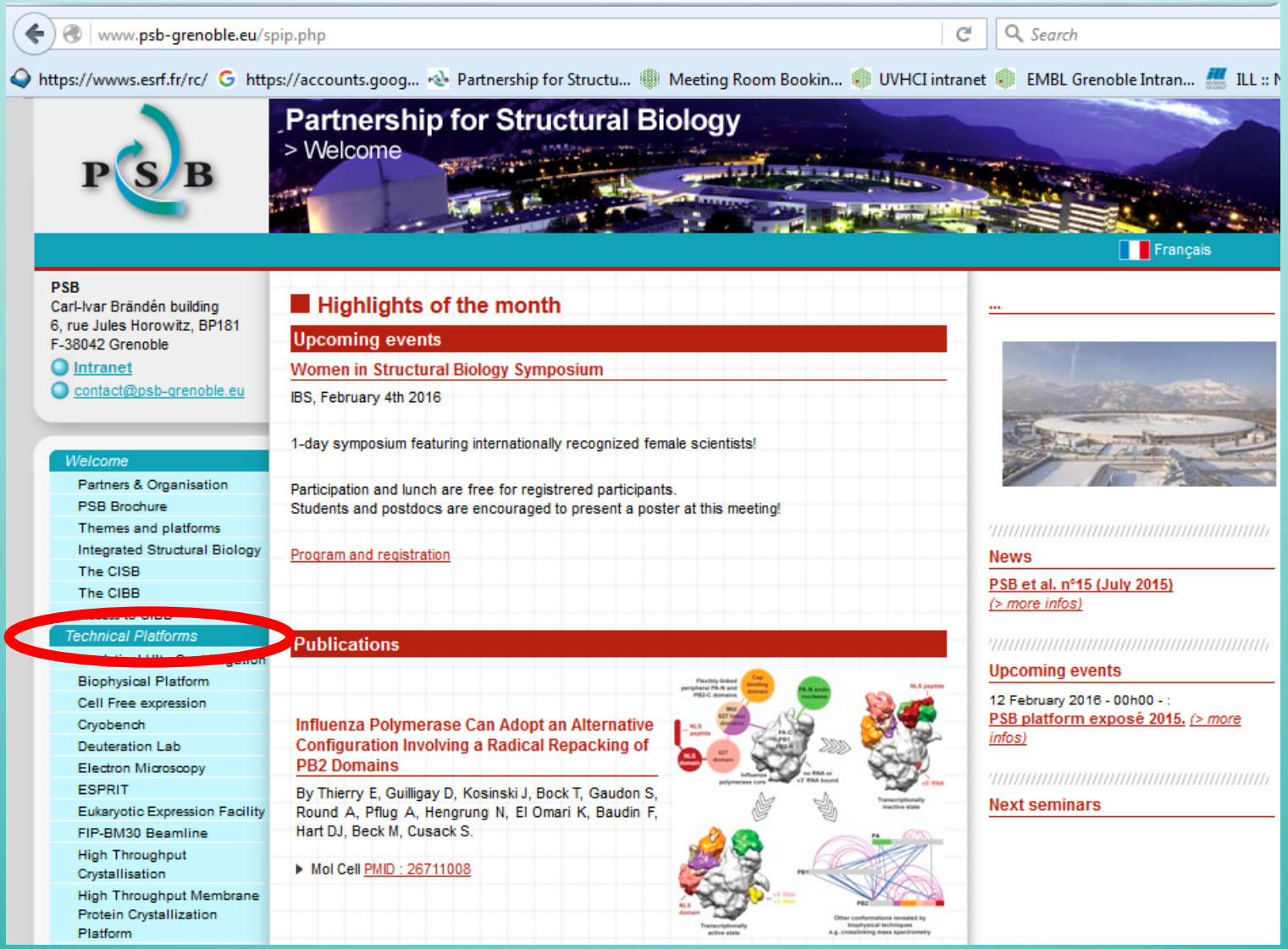
  ESRF: 1st March 2016
  ILL: 9th February 2016

- Transnational access (INSTRUCT, i-NEXT, BIO-NMR,…)

- Proprietary/paid-for access mode

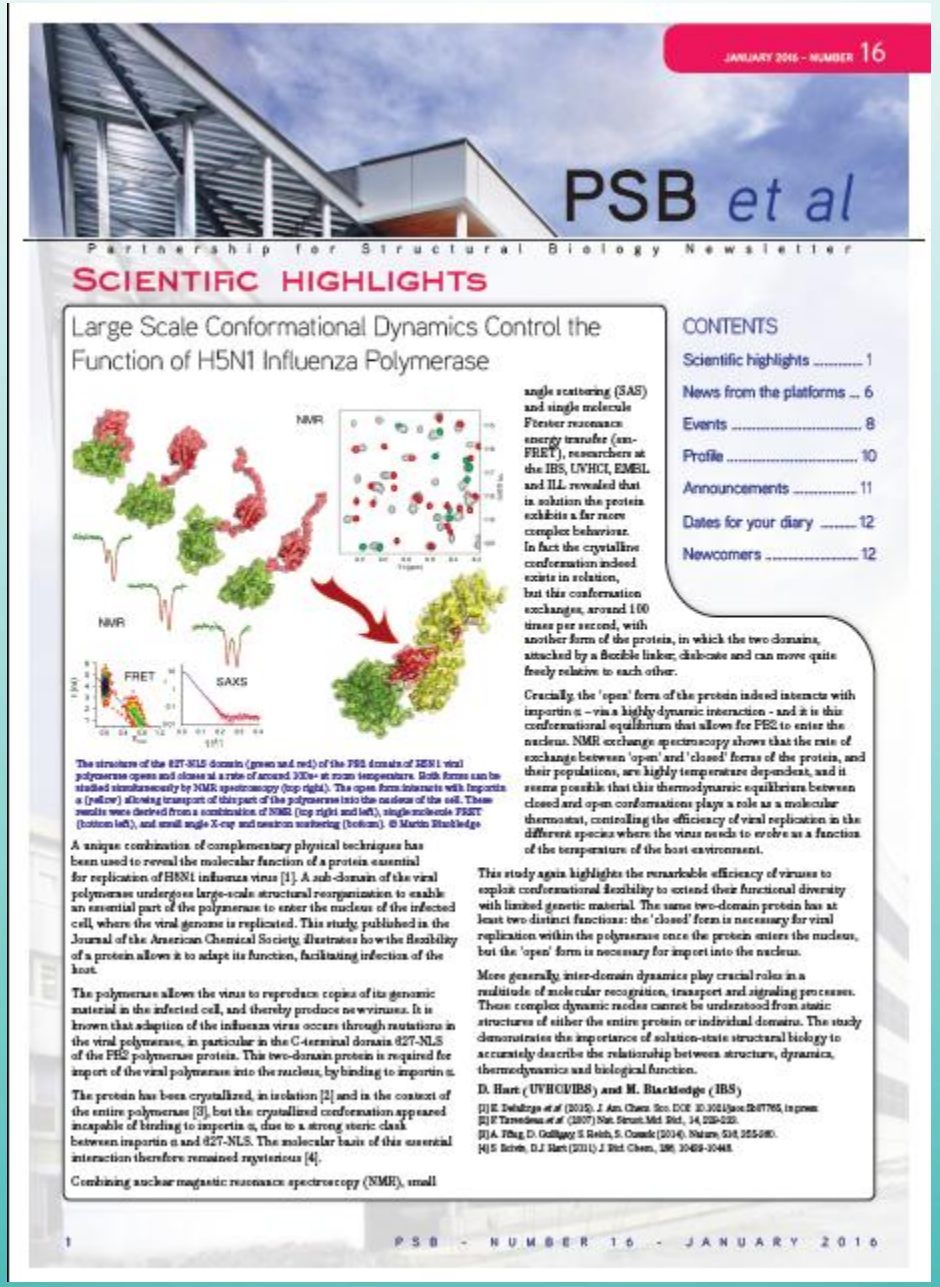
- Collaboration

www.psb-grenoble.eu



florent.bernaudat@esrf.fr

PSB Newsletter



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PSB *et al*

Partnership for Structural Biology Newsletter

SCIENTIFIC HIGHLIGHTS

Large Scale Conformational Dynamics Control the Function of H5N1 Influenza Polymerase

The structure of the 627-NLS domain (green and red) of the PB2 domain of H5N1 viral polymerase opens and closes at a rate of around 100s^{-1} at room temperature. Both forms can be studied simultaneously by NMR spectroscopy (top right). The open form interacts with importin α (yellow) allowing transport of this part of the polymerase into the nucleus of the cell. These results were derived from a combination of NMR (top right and left), single-molecule FRET (bottom left), and small angle X-ray and neutron scattering (bottom). © Martin Blackledge

A unique combination of complementary physical techniques has been used to reveal the molecular function of a protein essential for replication of H5N1 influenza virus [1]. A sub-domain of the viral polymerase undergoes large-scale structural reorganization to enable an essential part of the polymerase to enter the nucleus of the infected cell, where the viral genome is replicated. This study, published in the Journal of the American Chemical Society, illustrates how the flexibility of a protein allows it to adapt its function, facilitating infection of the host.

The polymerase allows the virus to reproduce copies of its genomic material in the infected cell, and thereby produce new viruses. It is known that adaption of the influenza virus occurs through mutations in the viral polymerase, in particular in the C-terminal domain 627-NLS of the PB2 polymerase protein. This two-domain protein is required for import of the viral polymerase into the nucleus, by binding to importin α.

The protein has been crystallized, in isolation [2] and in the context of the entire polymerase [3], but the crystallized conformation appeared incapable of binding to importin α, due to a strong steric clash between importin α and 627-NLS. The molecular basis of this essential interaction therefore remained mysterious [4].

Combining nuclear magnetic resonance spectroscopy (NMR), small angle scattering (SAS) and single molecule Förster resonance energy transfer (smFRET), researchers at the IBS, UVHCI, EMBL and ILL revealed that in solution the protein exhibits a far more complex behaviour. In fact the crystalline conformation indeed exists in solution, but this conformation exchanges, around 100 times per second, with another form of the protein, in which the two domains, attached by a flexible linker, dislocate and can move quite freely relative to each other.

Crucially, the 'open' form of the protein indeed interacts with importin α – via a highly dynamic interaction - and it is this conformational equilibrium that allows for PB2 to enter the nucleus. NMR exchange spectroscopy shows that the rate of exchange between 'open' and 'closed' forms of the protein, and their populations, are highly temperature dependent, and it seems possible that this thermodynamic equilibrium between closed and open conformations plays a role as a molecular thermostat, controlling the efficiency of viral replication in the different species where the virus needs to evolve as a function of the temperature of the host environment.

This study again highlights the remarkable efficiency of viruses to exploit conformational flexibility to extend their functional diversity with limited genetic material. The same two-domain protein has at least two distinct functions: the 'closed' form is necessary for viral replication within the polymerase once the protein enters the nucleus, but the 'open' form is necessary for import into the nucleus.

More generally, inter-domain dynamics play crucial roles in a multitude of molecular recognition, transport and signaling processes. These complex dynamic modes cannot be understood from static structures of either the entire protein or individual domains. The study demonstrates the importance of solution-state structural biology to accurately describe the relationship between structure, dynamics, thermodynamics and biological function.

D. Hart (UVHCI/IBS) and M. Blackledge (IBS)

[1] E. Deblingo *et al.* (2015). J. Am. Chem. Soc. DOI: 10.1021/jacs.5b07765, in press.
[2] F. Tarendeau *et al.* (2007) Nat. Struct. Mol. Biol., 14, 229-233.
[3] A. Pflug, D. Guilligay, S. Reich, S. Cusack (2014). Nature, 516, 355-360.
[4] S. Resa, D.J. Hart (2011) J. Biol. Chem., 286, 10459-10465.


Thank you for your attention