

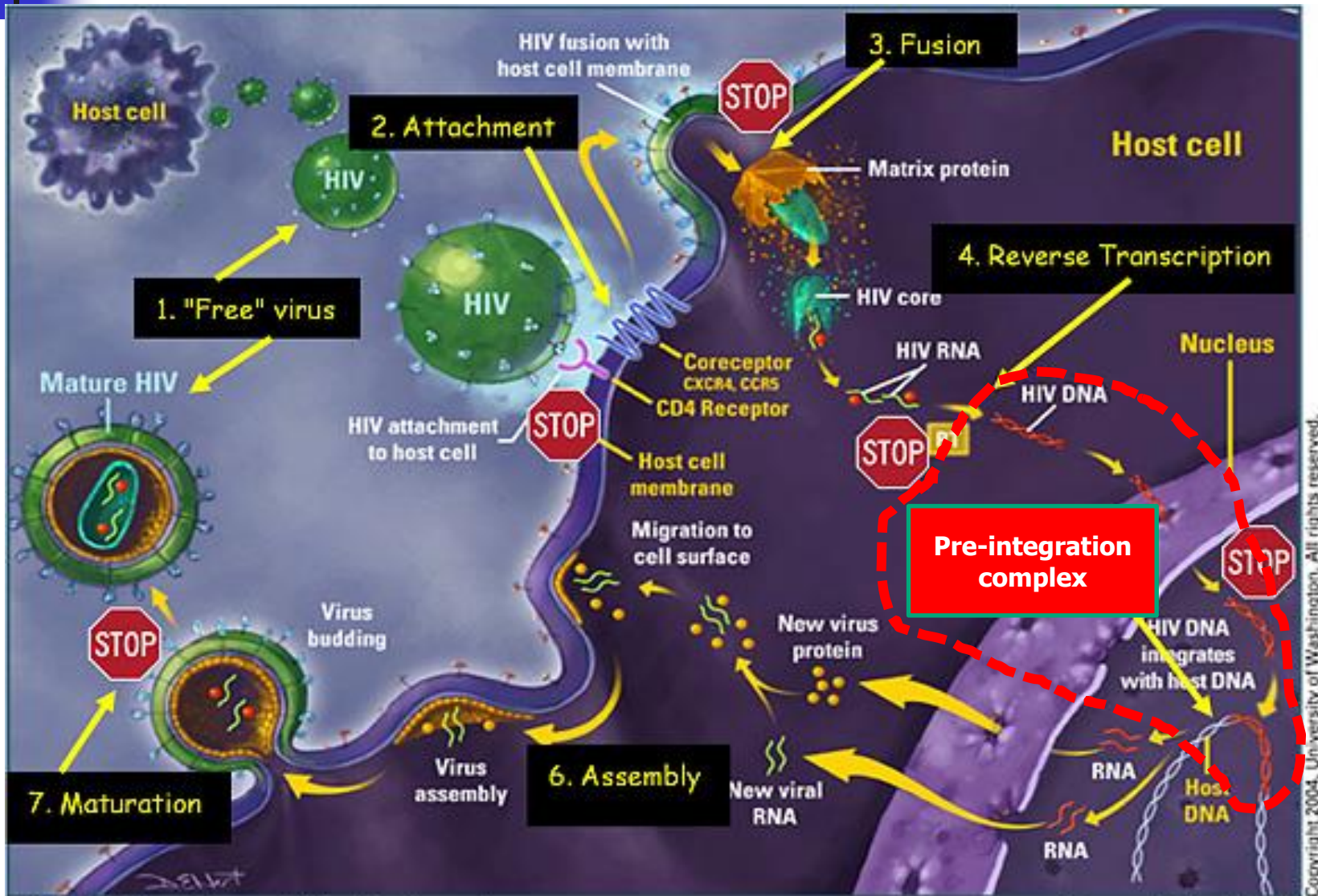


STRUCTURAL STUDIES OF THE HIV-1 PRE-INTEGRATION COMPLEX – IN/LEDGF INTERACTION INHIBITORS

Marc Ruff
Laboratory of Integrated structural biology
IGBMC, Illkirch, France

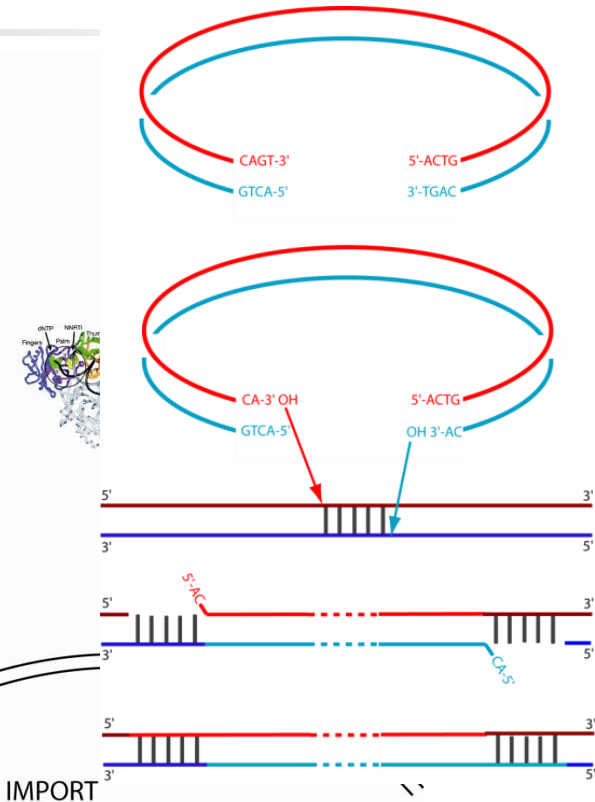
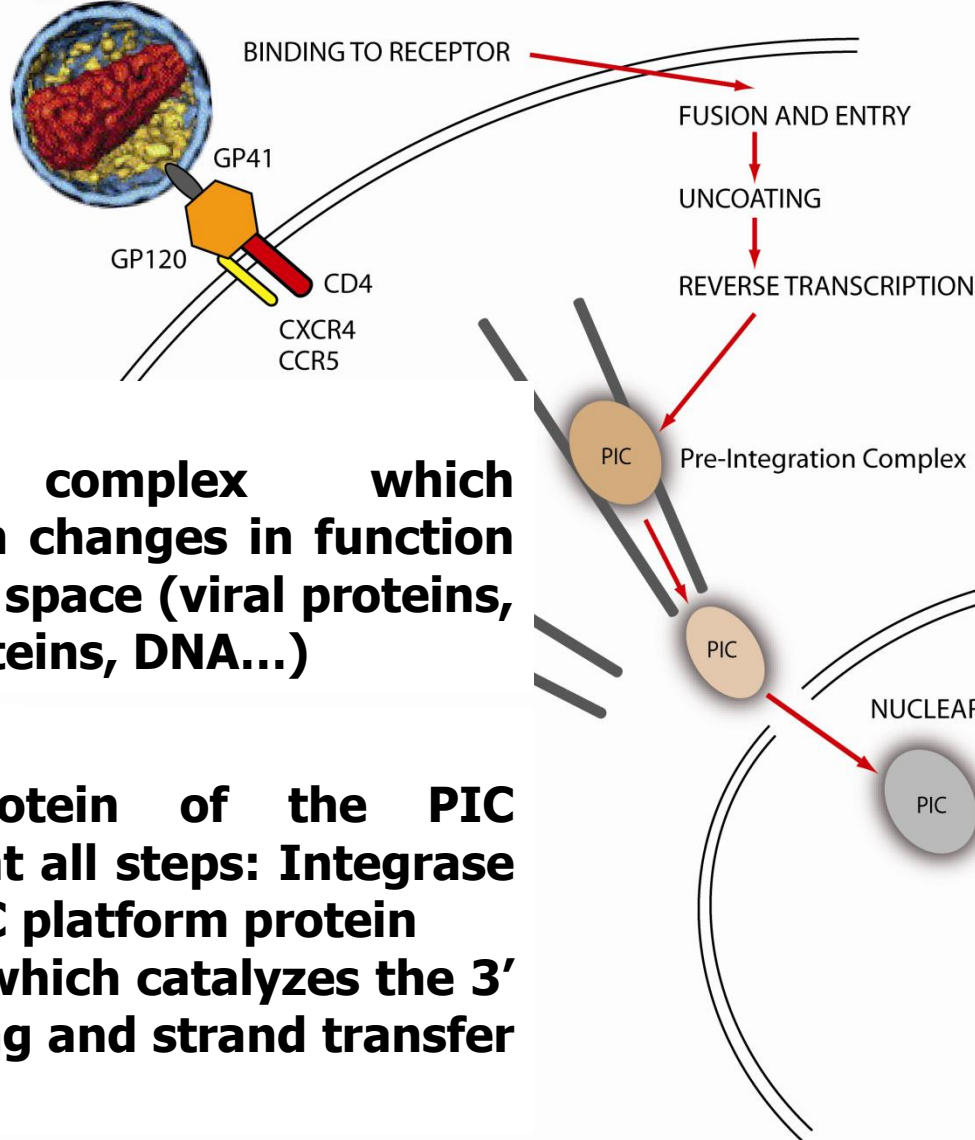
ruff@igbmc.fr

Schematic diagram of HIV multiplication cycle



HIV-1 pre-integration complex

Briggs et al., 2006



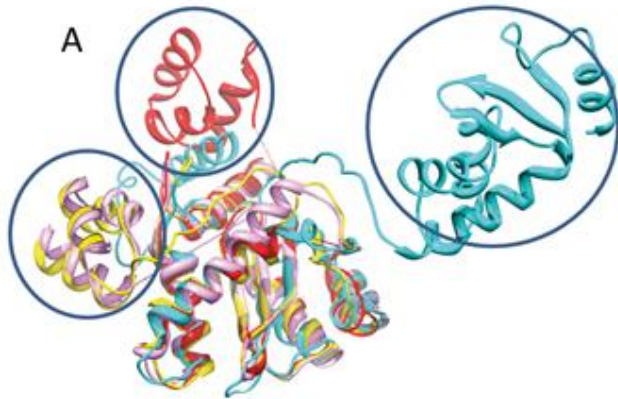
PIC:

Dynamic complex which composition changes in function of time and space (viral proteins, cellular proteins, DNA...)

Integrase:

- **Core protein of the PIC present at all steps: Integrase is the PIC platform protein**
- **Enzyme which catalyzes the 3' processing and strand transfer reaction**

Integrase flexibility



RSV, SIV, PFV, HIV-1 structures show different orientation of the **N-terminal domain**



PFV, HIV1, HIV2 structures show different orientation of the **C-terminal domain**

High flexibility between domains → Integrase is a flexible protein (IDP)
Protein with multiple structures and multiple functions

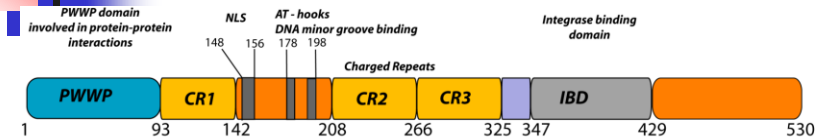
Partners and/or Post Translational Modifications (PTMs) are needed to stabilize functional conformations

Structural and functional studies

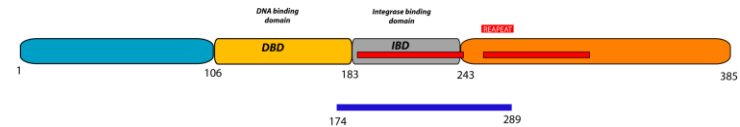
Isolate stable IN complexes
(VBP1, TRN-SR2, LEDGF, INI1)

Isolate protein with PTMs
(Production in eukaryotic cells)

IN/LEDGF/INI1 : complex formation and purification (*E. Coli*)



LEDGF



INI1

HIS-INI1(174-289)

His Affinity

Gel filtration G200

High salt, detergent

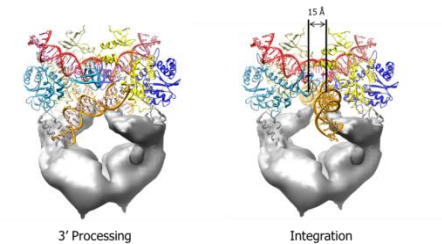
+ IN/LEDGF

High salt, detergent

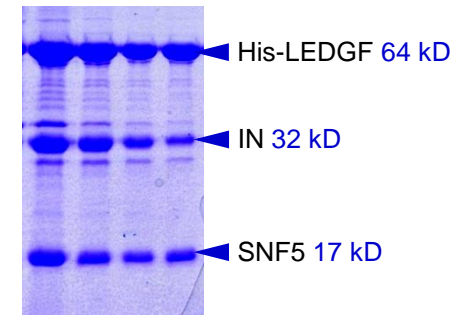
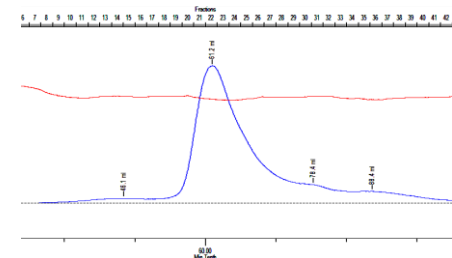
Complex formation
by dialysis

Gel filtration G200

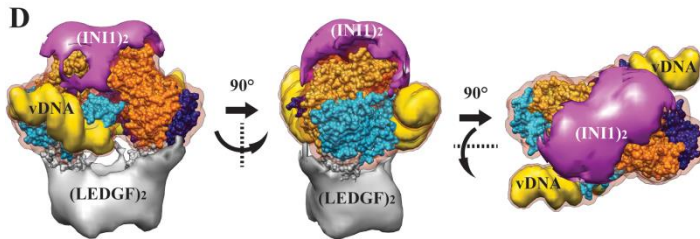
CryoEM reconstruction and atomic
model flexible fitting (NMFF)



Michel et al,(2009)
EMBO J, 28, 980-991

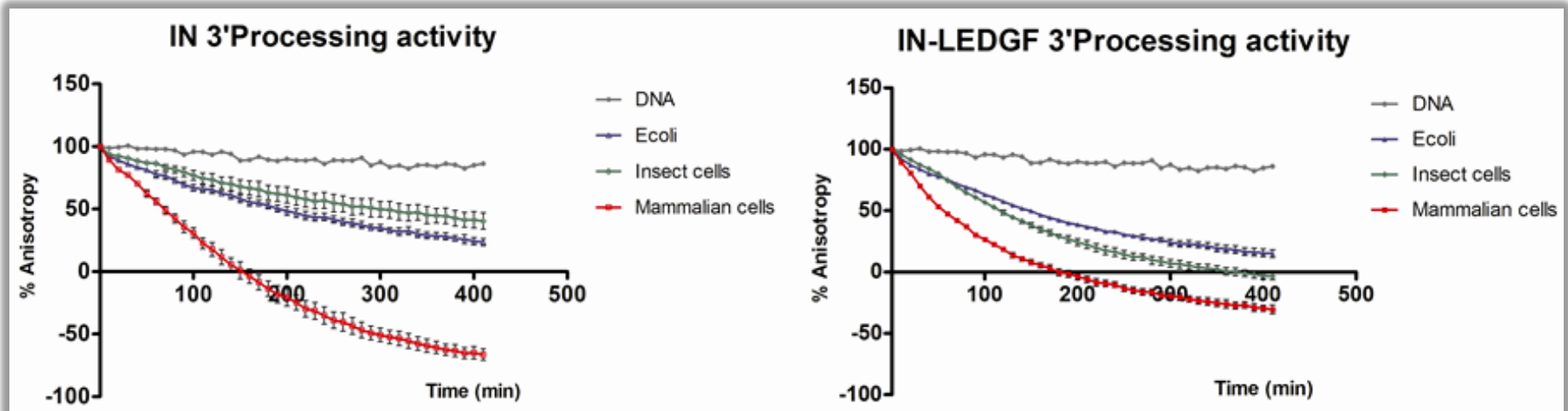
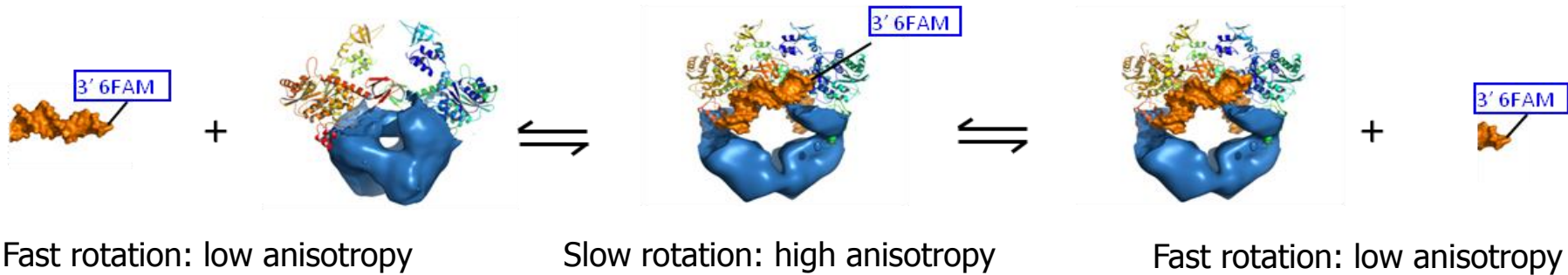


Maillot B et al,(2013) PLoS ONE, e60734



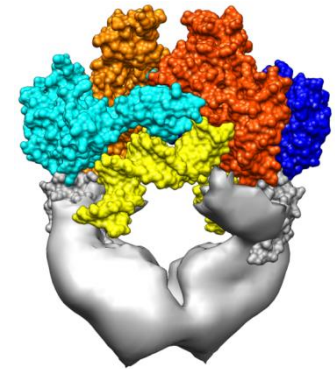
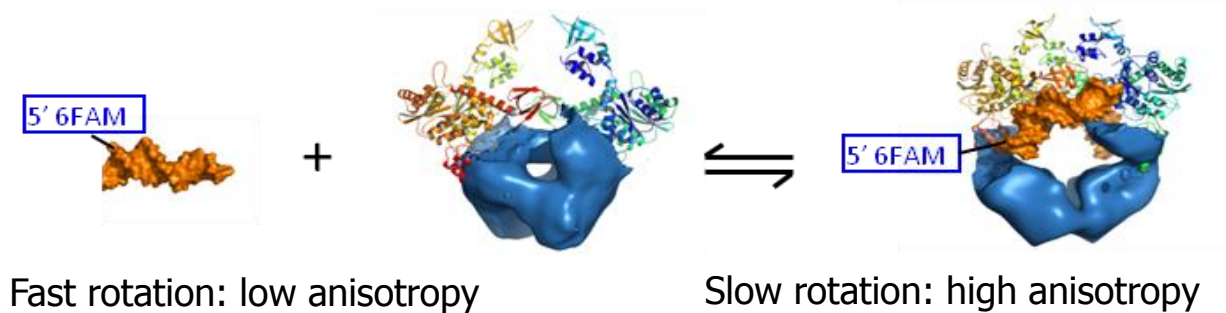
Yield: 2.5 mg of complex (INT 0.5L, LEDGF 0.5L, SNF5 1.0L)

Protein produced in E Coli, Insect and mammalian cells



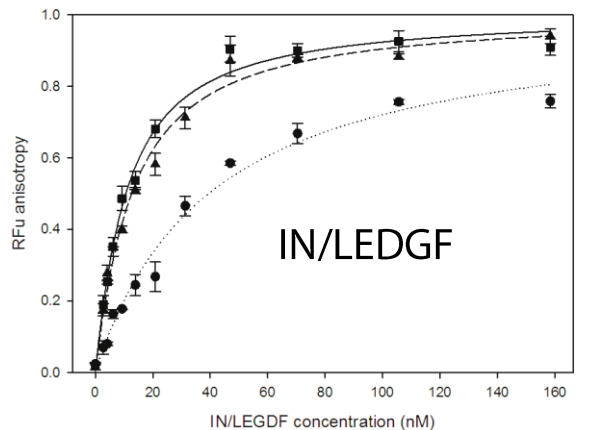
**Production in mammalian cells:
Increase activity, solubility and presence of PTM (phosphorylation and acetylation)**

Functional and structural studies: Viral DNA Binding

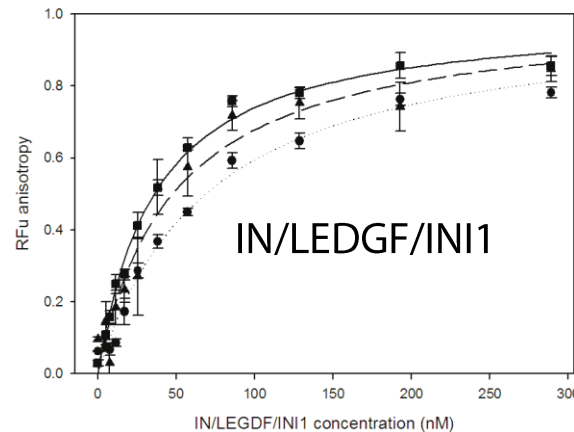


IN/LEDGF

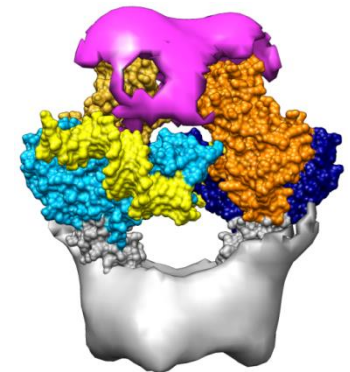
Maillot et al., PLOS ONE, 2013



$K_d = 10.6 \pm 0.5 \text{ nM}$



$K_d = 35 \pm 4 \text{ nM}$

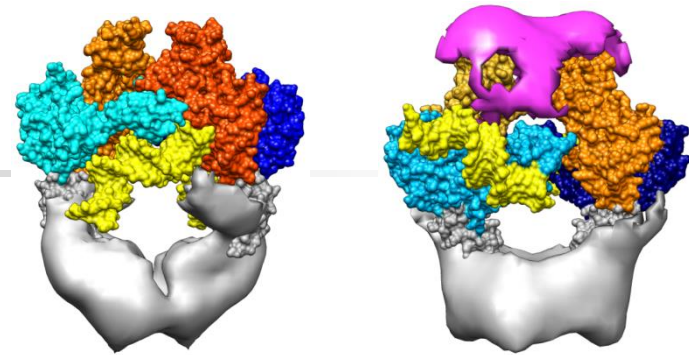


IN/LEDGF/INI1

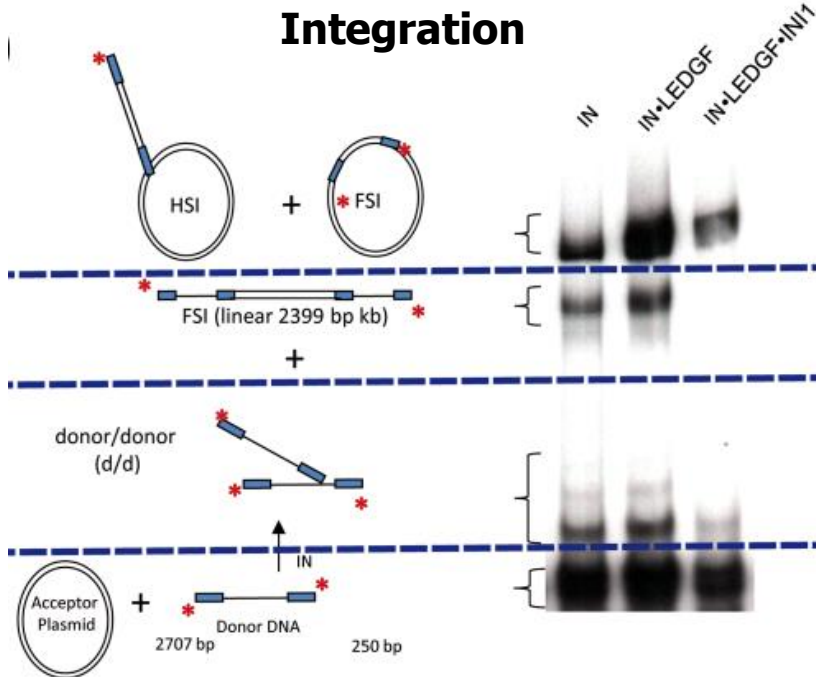
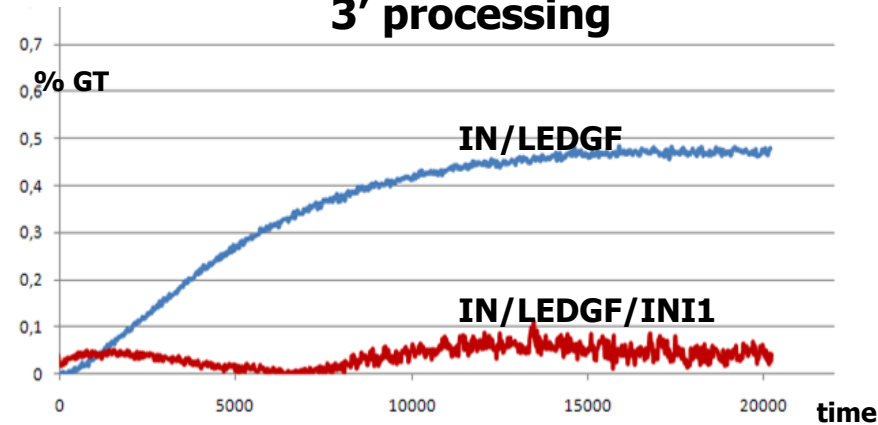
Michel et al. EMBO J. 2009

IN/LEDGF complexes, with and without INI1, specifically bind the U5 DNA with similar binding constants

Functional and structural studies: 3' processing and integration



3' processing



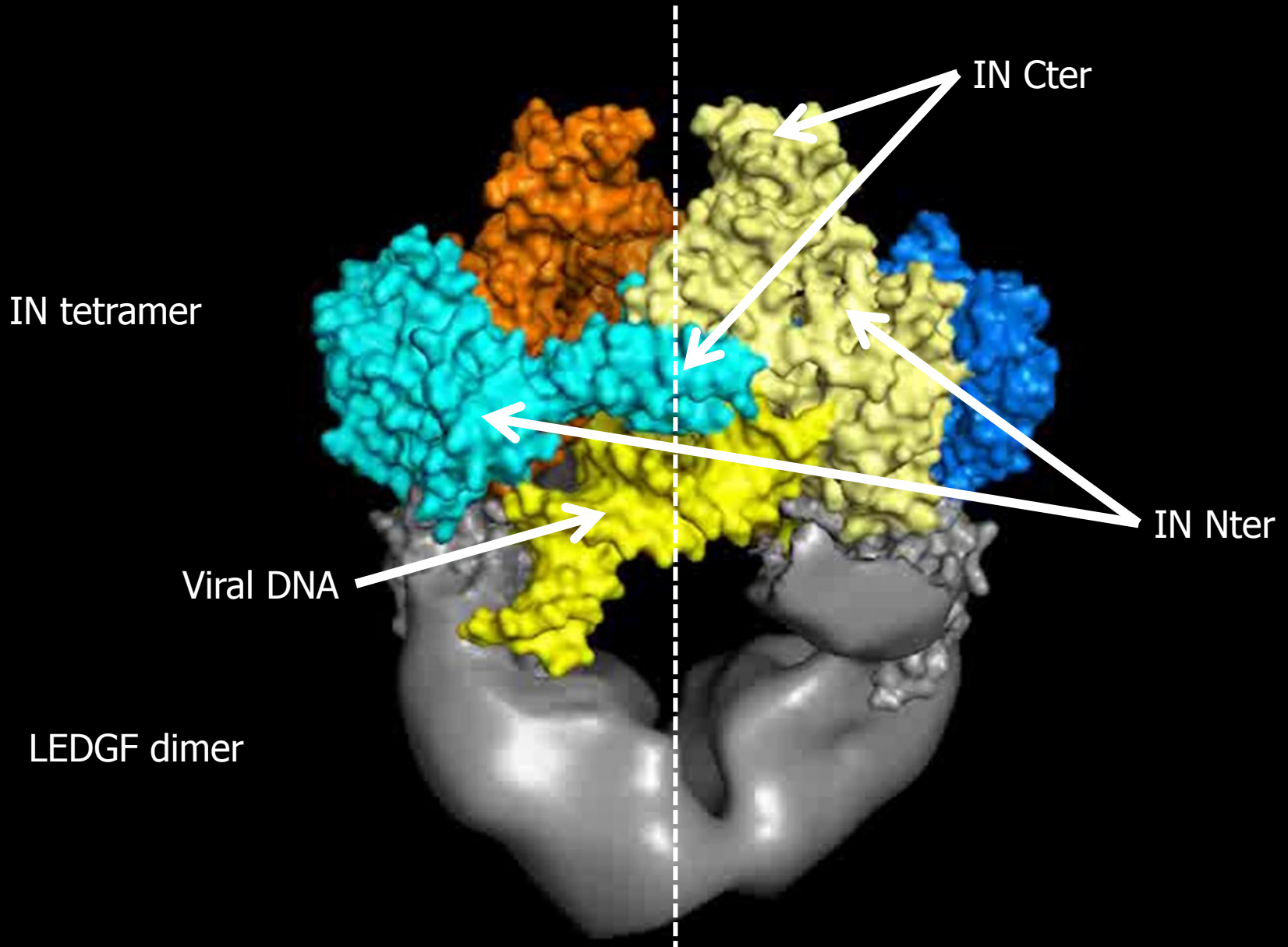
INI1 inhibits the 3' processing activity but not integration



INI1 stabilizes the PIC by maintaining integrase in a stable constrained conformation preventing non-specific interactions and auto integration

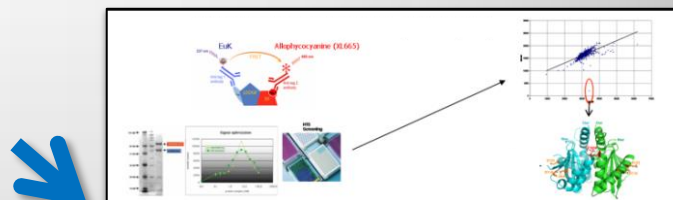
LEDGF organizes and stabilizes an active integrase tetramer suitable for specific vDNA integration.

IN/LEDGF/vDNA complex



Protein – Protein interaction and allosteric inhibitors

HTP IN – LEDGF interaction
inhibitors screening (HTRF)



IN CCD Production and
crystallization

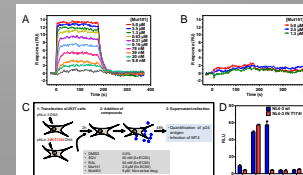
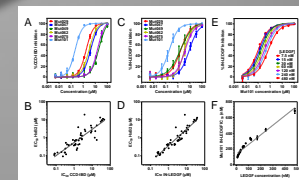


Inhibitors crystal soaking, data
collection, structure

Structure based drug design

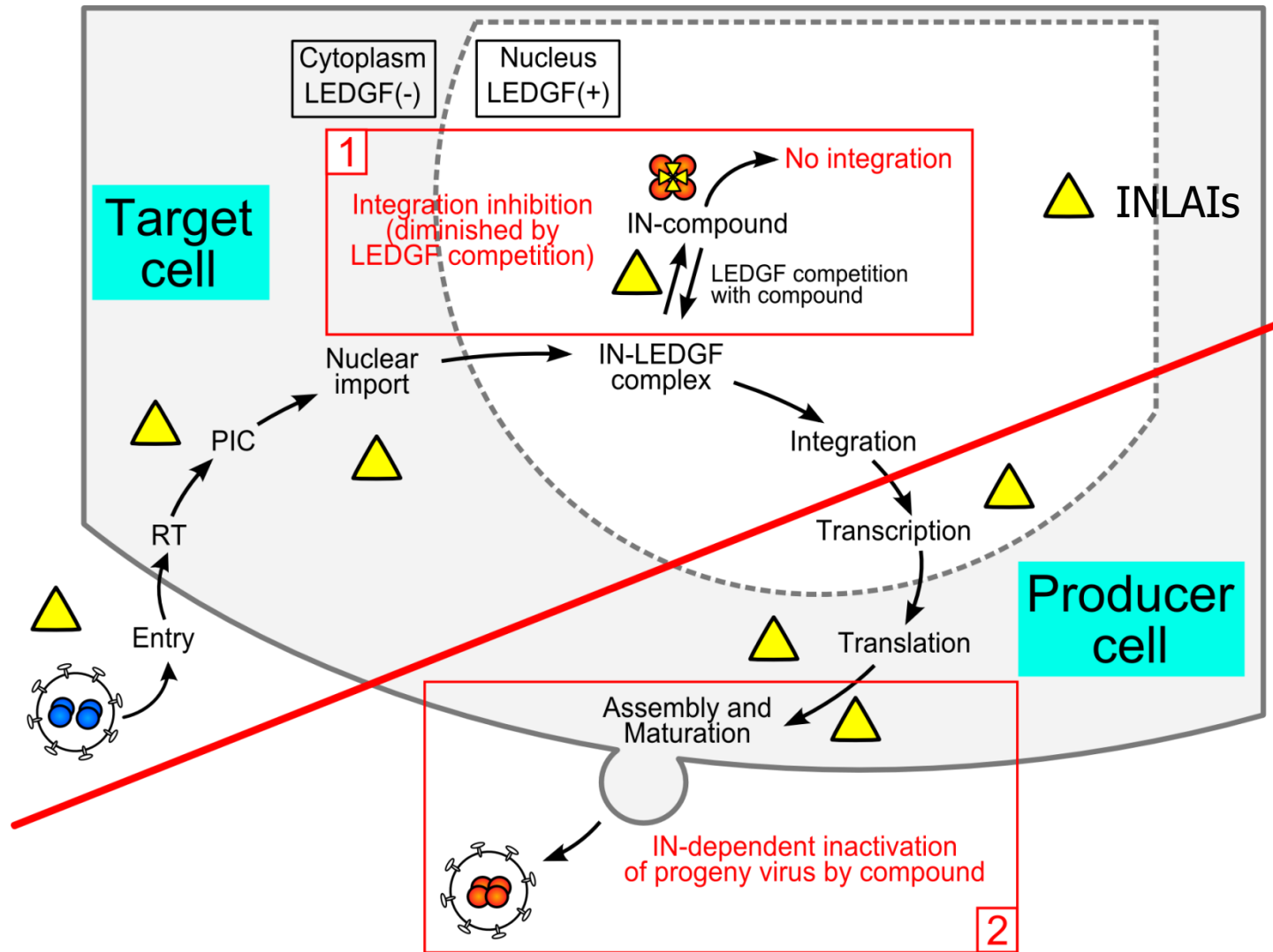
in-vitro functional assay

In cellulo functional assay

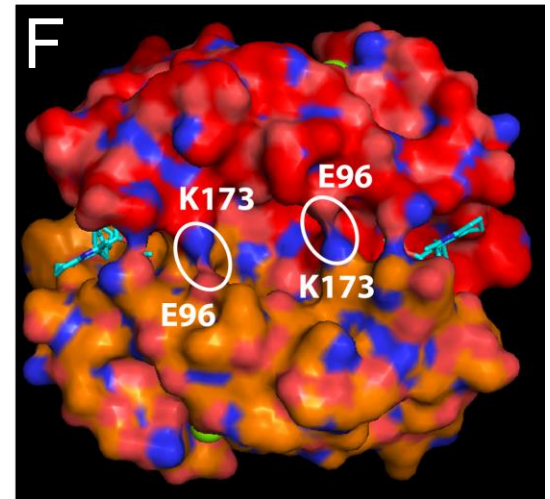
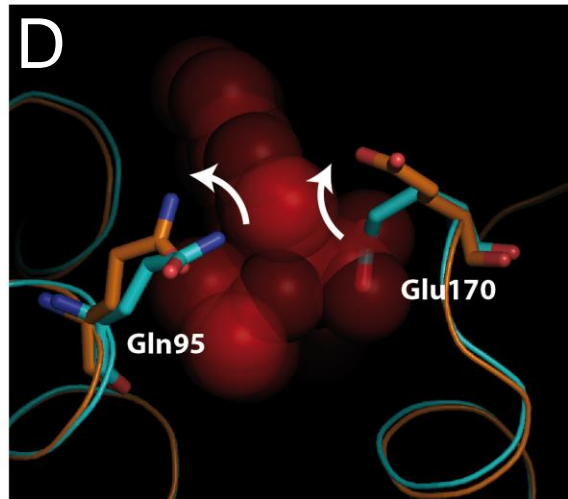
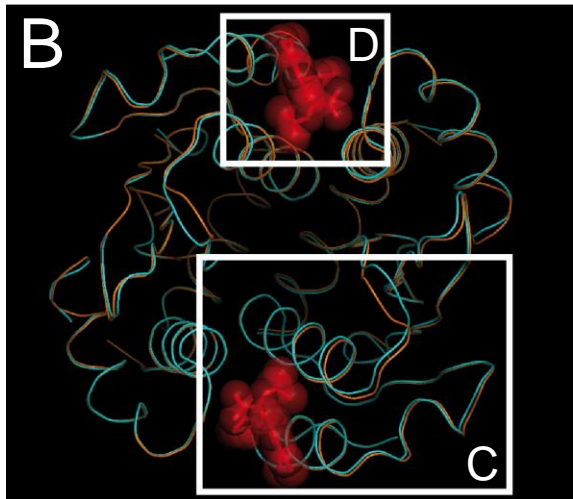
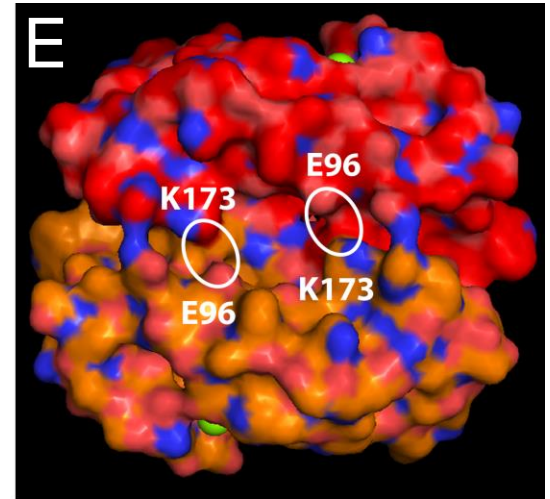
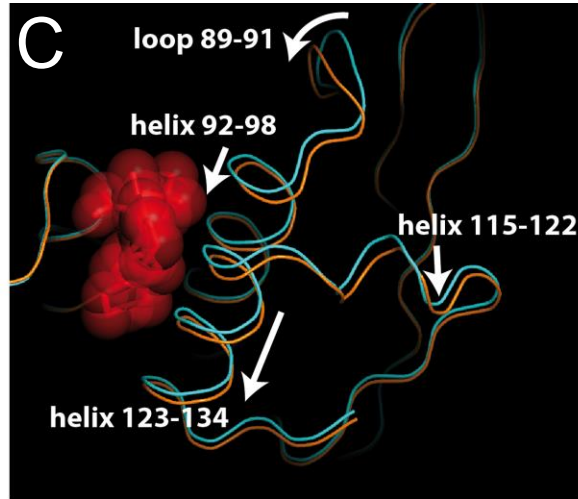
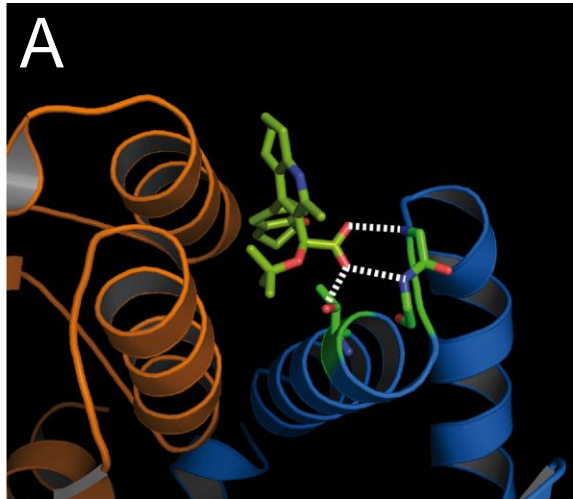


**Inhibition of integration and
viral maturation in late phase
→ Allosteric Inhibitor**

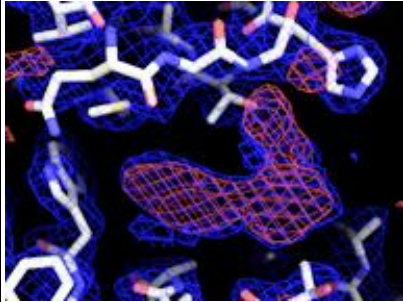
IN – LEDGF interaction and IN allosteric inhibitors



Structure comparison of HIV-1 Integrase Catalytic core Domain with and without MUT101



INLAIs inhibitors



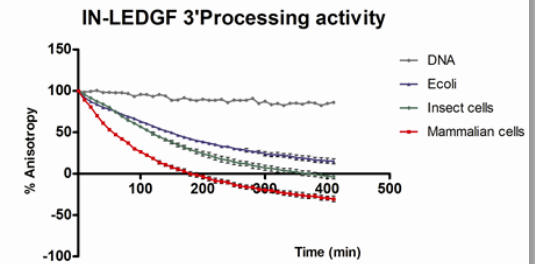
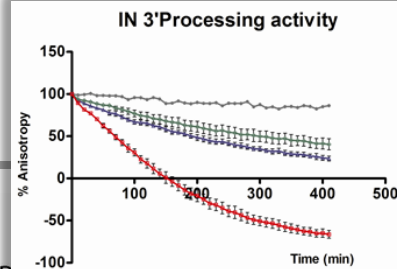
~70 structures solved

IN/LEDGF interaction and allosteric inhibitors. Ligand binding lead to structural changes in the active site, dimeric interface, accessible surface.

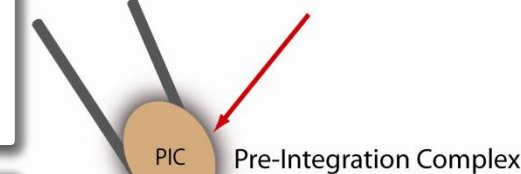
Mammalian cells production: increase integrase stability, solubility and 3'processing activity. Presence of acetylation and phosphorylation

LEDGF stabilizes an IN tetramer and increases its integration and 3'processing activity

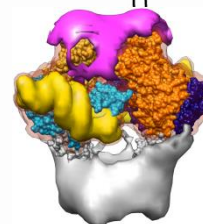
INI1 prevents non specific aggregation and auto integration on the way to nucleosomes in the nucleus



FUSION AND ENTRY
UNCOATING
REVERSE TRANSCRIPTION



Integrase
VBP1
TRN-SR2



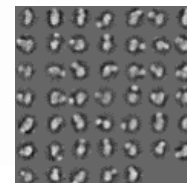
Integrase
LEDGF
TRN-SR2

Integrase
LEDGF
INI1

NUCLEAR IMPORT

PIC

3' PROCESSING



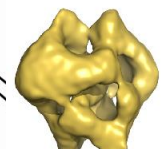
Integrase
LEDGF
DNA

INTEGRATION

Integrase
LEDGF
Nucleosome



Integrase
LEDGF



Acknowledgments

Integrative Structural Biology, IGBMC, Illkirch

Sylvia Eiler
Nicolas Levy
Karine Pradeau
Robert Drillien
Damola Oladosu

Group of Molecular Electron Microscopy, IGBMC, Illkirch

Patrick Schultz
Corinne Crucifix

Mutabilis, France

Richard Benarous, François Moreau

Infection Diseases Department, Cochin Institute, Paris

Stéphane Emiliani

Fundamental Microbiology and Pathogenicity, University of Bordeaux 2

Vincent Parissi

ENS, CNRS, Pasteur Institute, Lyon

Marc Lavigne

Functional Genomics and Cancer, IGBMC, Illkirch

Ali Hamiche Catherine Romain

Faculty of pharmacy, University of Strasbourg, Illkirch

Yves Mely

Institute of cellular and molecular Biology, Strasbourg,

Sylviane Muller, François Stricher

CovalX, Technoparkstrasse, 1, CH-8005, Zürich, Switzerland

Alexis Nazabal

Bio-organic Mass Spectrometry Laboratory, University of Strasbourg, Cronembourg

Sarah Sanglier-Cianferani
Sarah Lennon

Members of the IGBMC Mass Spectrometry Facility

**Members of Structural Biology and Genomics platform
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