IGBMC
Institut de génétique et de biologie moléculaire et cellulaire

STRUCTURAL AND FUNCTIONAL STUDIES OF THE HIV-1 PRE-INTEGRATION COMPLEX

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Retrovirus genera

Use a reverse transcription step in the viral replication cycle

Genus	Examples of virus	Host
Alpharetrovirus	Rous sarcoma virus	Chickens
Betaretrovirus	Mouse mammary tumor virus	Mice
Gammaretrovirus	Murine leukemia virus	Mice
Deltaretrovirus	Human T cell leukemia virus type 1	Humans
Epsilonretrovirus	Walleye dermal sarcoma virus	Fish
Lentivirus	Human immunodeficiency virus type 1	Humans
	Simian immunodeficiency virus	Monkeys
	Feline immunodeficiency virus	Cats
Spumavirus	Simian foamy virus	Monkeys

Lentivirus

Lentivirus

From Latin *lentis* (slow), for slow progression of disease

VIRION

Spherical enveloped particle.

Diameter 100 nm.

Conical capsid.

GENOME

Linear ss RNA, positive sense.

Two identical segments, each 9.3 Kb.

Cellular $tRNA^{lys3}$ molecules packaged in virions used as primers for reverse transcription.

GENES AND PROTEINS

Four capsid proteins: MA (p17), CA (p24), NC (p7), p6.

Three enzymes: PR (p10), RT (p51/66), IN (p32).

Two envelope proteins: SU (gp120) and TM (gp41).

Six regulatory proteins: Vif, Vpu, Vpr, Tat, Rev, Nef.

Human Immunodefiency Virus

VIRUSES AND HOSTS

Human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2).

Simian immunodeficiency virus.

Equine, bovine, feline immunodeficiency viruses.

DISEASES

Acquired immune deficiency syndrome (AIDS) first described in 1981.

A major global pandemic today (more than 44 million people infected).

HIV replicates in and kills lymphocytes and macrophages.

Results in depletion of CD4+ T cells to render host immune-incompetent.

As a result, opportunistic infections by other pathogens are often fatal.

HIV is transmitted through sexual contact and blood exchange.

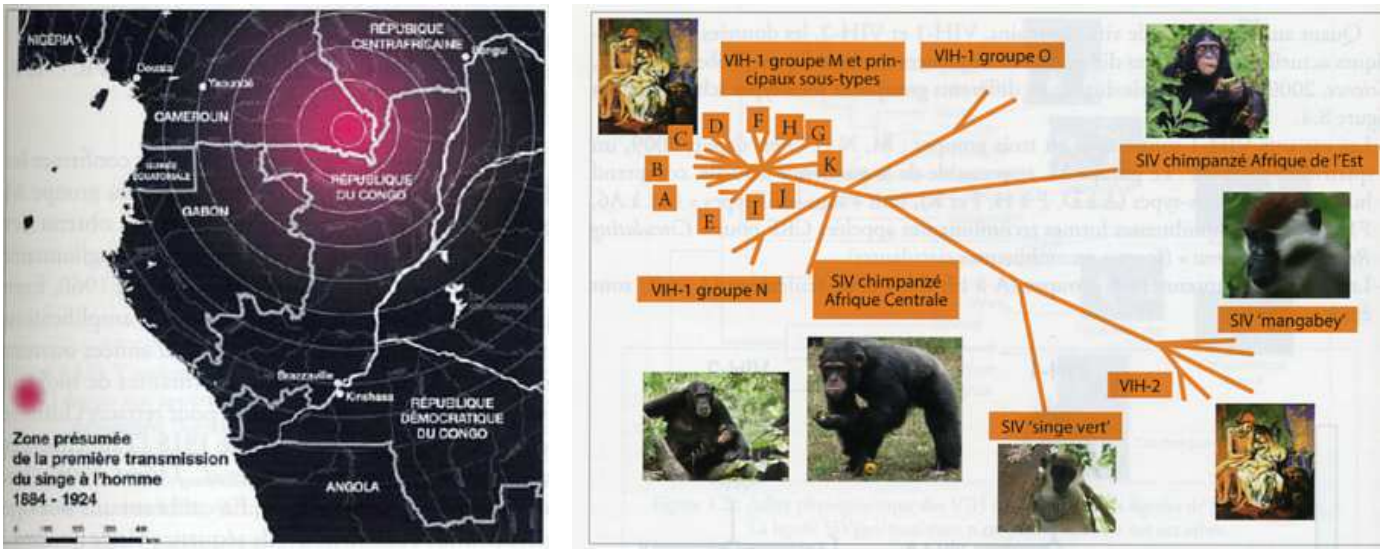
DISTINCTIVE CHARACTERISTICS

Proviral DNA can enter nucleus without requirement for cell division.

Lentiviruses make a complex set of singly and doubly spliced mRNAs.

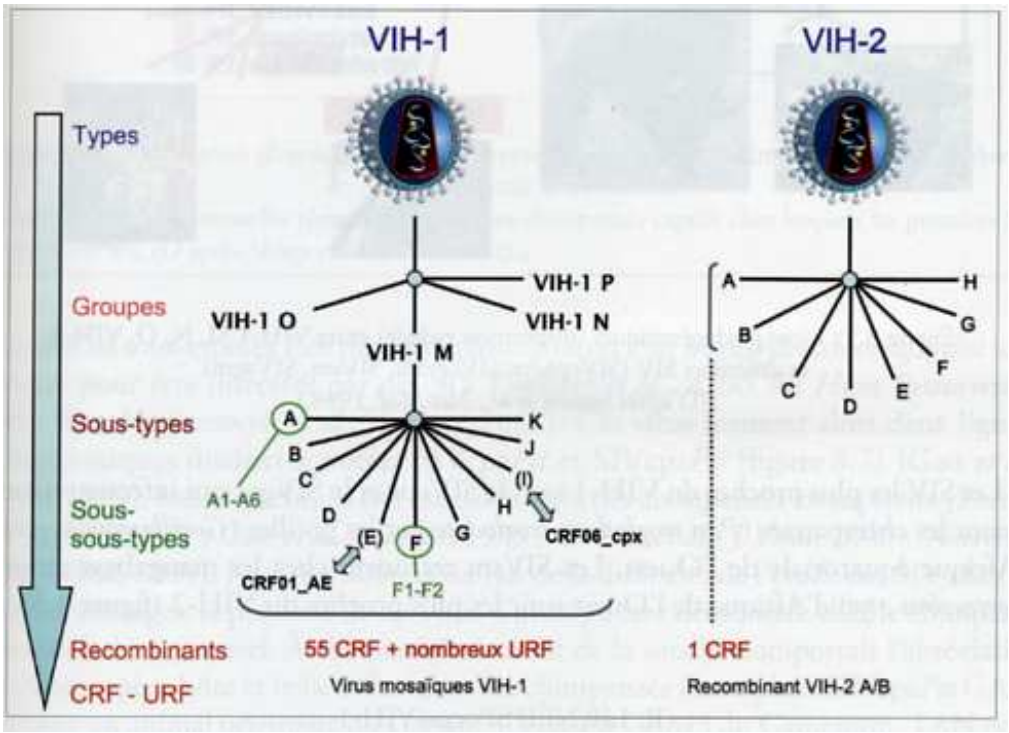
Six regulatory proteins control virus production and pathogenesis.

HIV: origin of the pandemia



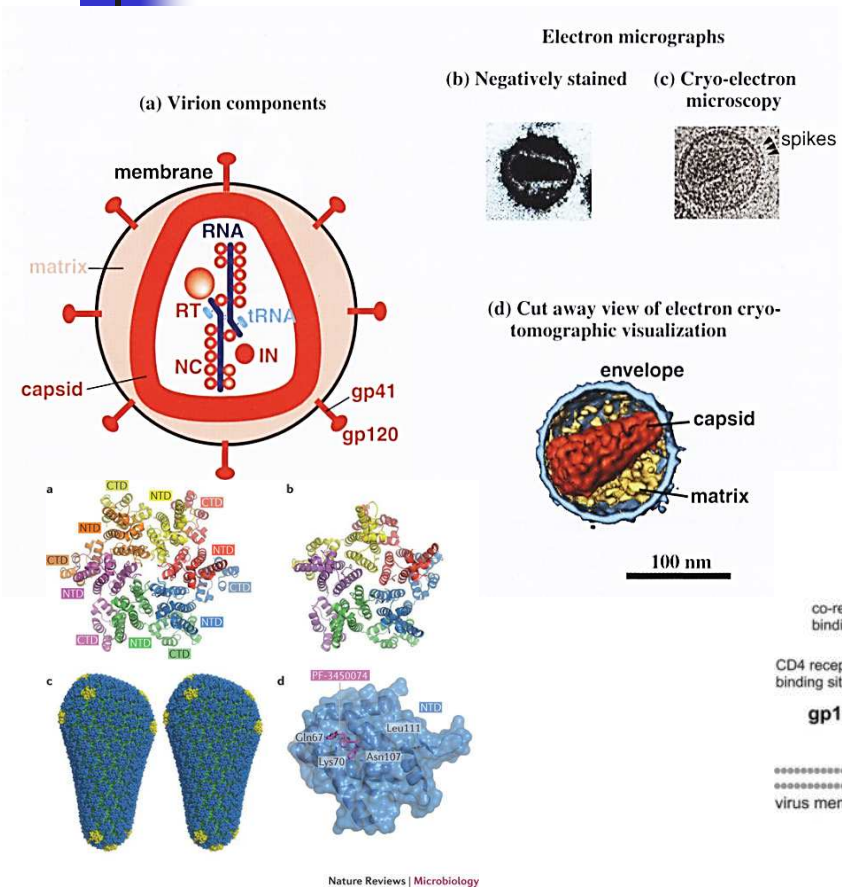
François Charles Javaugue, VIH, ed. Hermann, 2014

HIV phylogeny

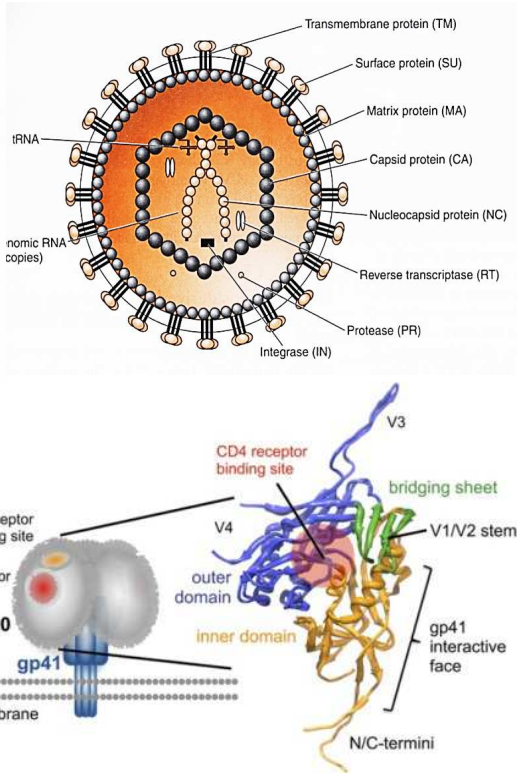


François Charles Javaugue, VIH, ed. Hermann, 2014

Virions components



Alan Engelman and Peter Cherepanov, Nature Review in Microbiology, 2012, 10, 279-289



Miklos Guttman et al., J Virol. Aug 2012; 86(16): 8750–8764

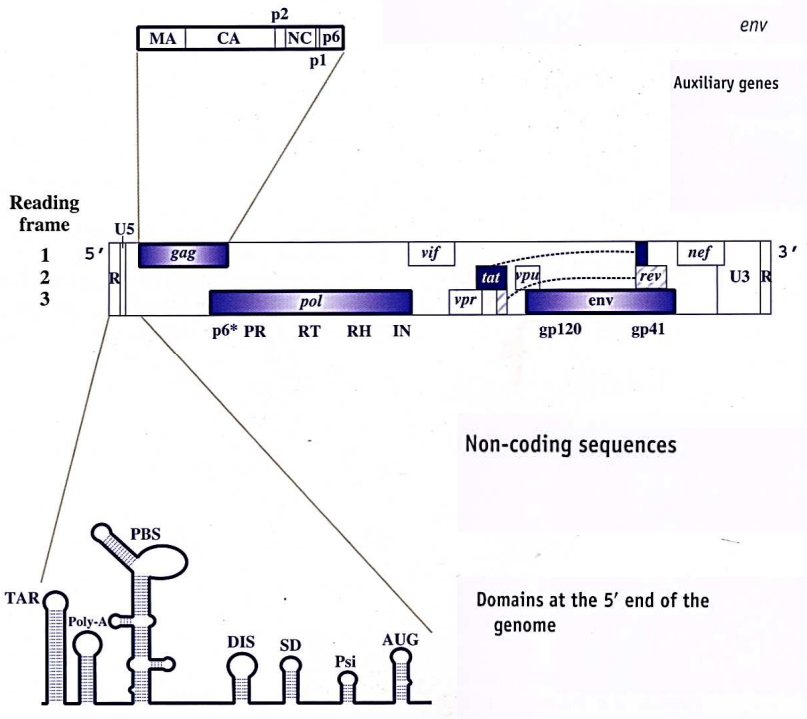
HIV-1 genome organization

Main genes

gag	*g*roup-specific *a*ntigen (encodes matrix, capsid, p2, nucleocapsid, p1 and p6)
pol	*pol*ymerase (encodes p6*, protease, reverse transcriptase, RNase H, integrase)
env	*env*elope

Auxiliary genes

nef	*n*egative regulatory *f*actor
rev	regulator of *e*xpression of *v*irion proteins
tat	*t*rans*a*ctivator of *t*ranscription
vif	*v*irion *i*nfectivity *f*actor
vpr	*v*iral *p*rotein *R*
vpu	*v*iral *p*rotein *U*



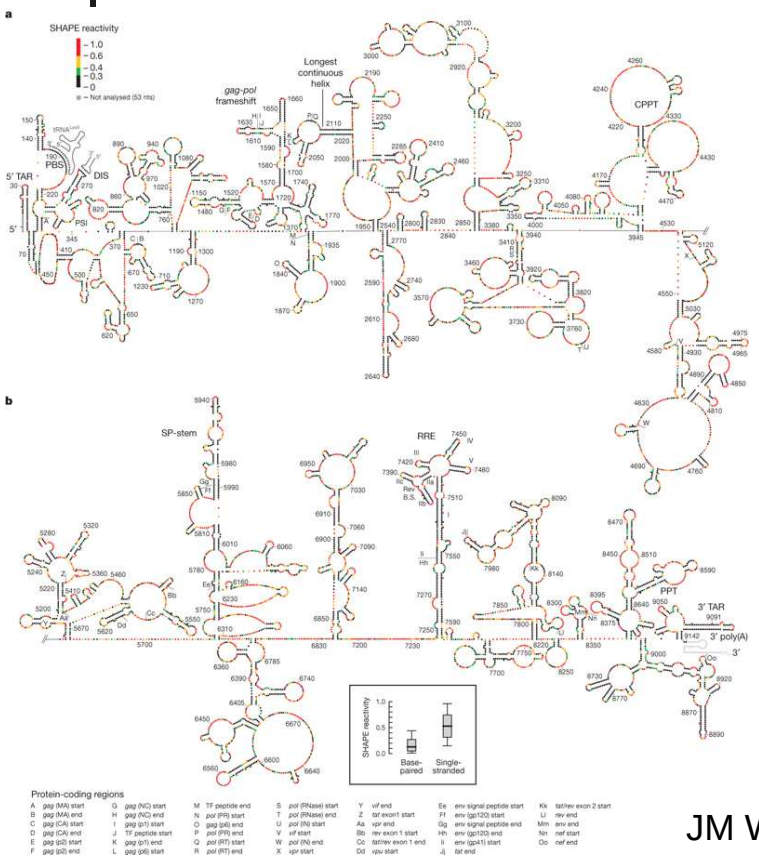
Non-coding sequences

R	repeat sequence
U3	unique sequence at 3′ end of genome
U5	unique sequence at 5′ end of genome

Domains at the 5′ end of the genome

TAR	trans-acting response element
Poly-A	polyadenylation signal
PBS	primer-binding site
DIS	dimerization initiation site (involved in formation of kissing loop complex)
SD	splice donor site
Psi (ψ)	main part of the packaging signal
AUG	start codon of the *gag* gene

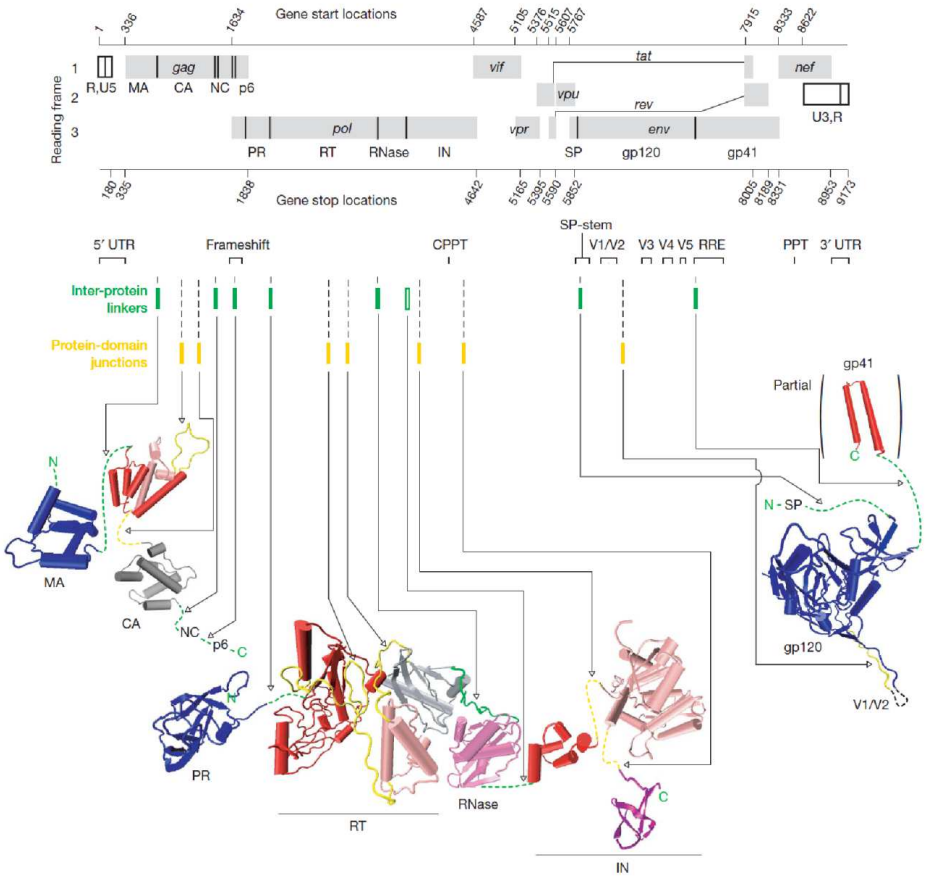
Structure of the HIV-1 NL4-3 genome



The 5' (**a**) and 3' (**b**) genome halves are shown. Nucleotides are coloured by their absolute SHAPE reactivities. Every nucleotide is shown explicitly as a sphere; base pairing is indicated by adjacent parallel orientation of the spheres. Protein domains are identified by letters; TF, transframe peptide; nts, nucleotides. Important structural landmarks are labelled explicitly. Intermolecular base pairs involving the $tRNA^{Lys3}$ primer and the genomic dimer are shown in grey. Inset shows a box plot illustrating SHAPE reactivities for single-stranded versus paired nucleotides in the model. Median reactivities are indicated by bold horizontal lines; the large box spans the central 50% of the reactivity data.

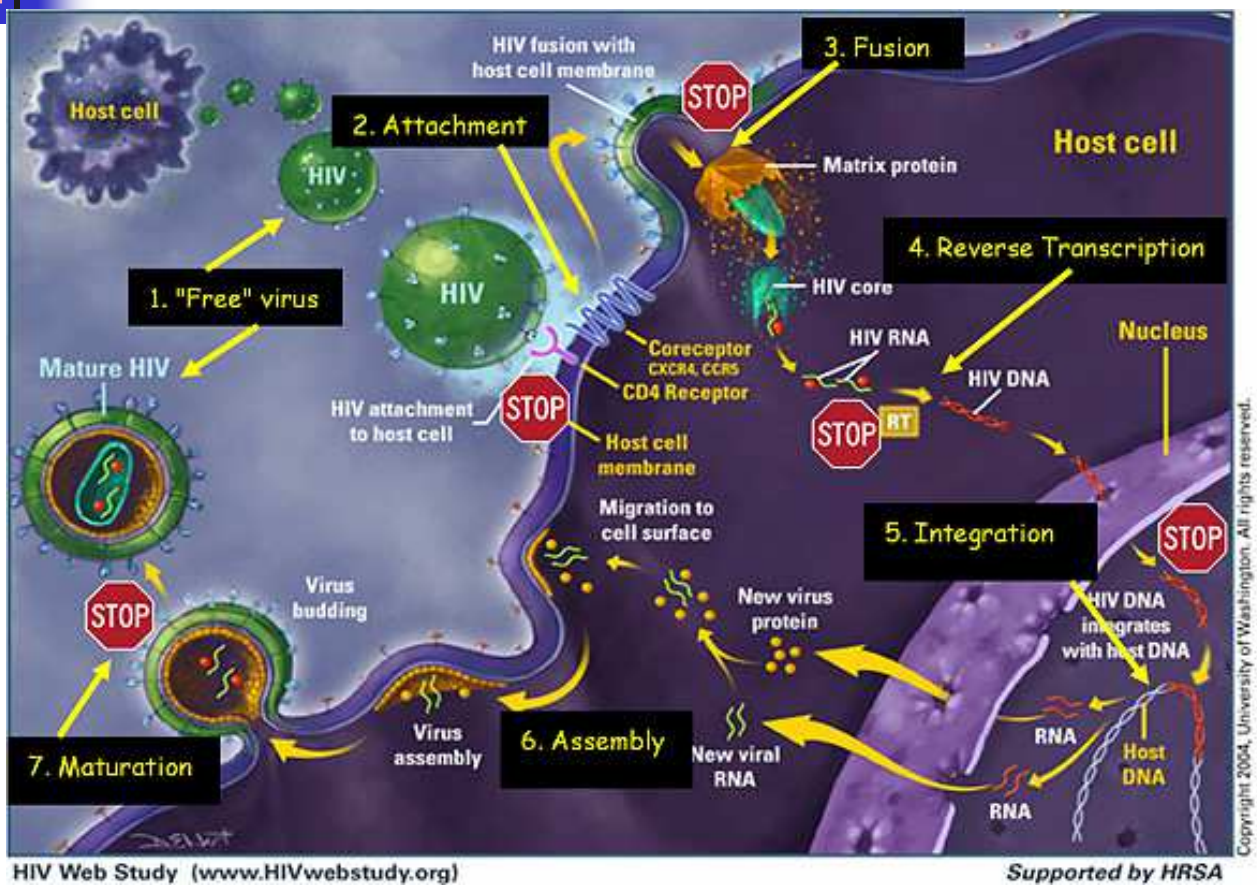
JM Watts *et al. Nature* **460**, 711-716 (2009)

HIV genome and viral protein structures

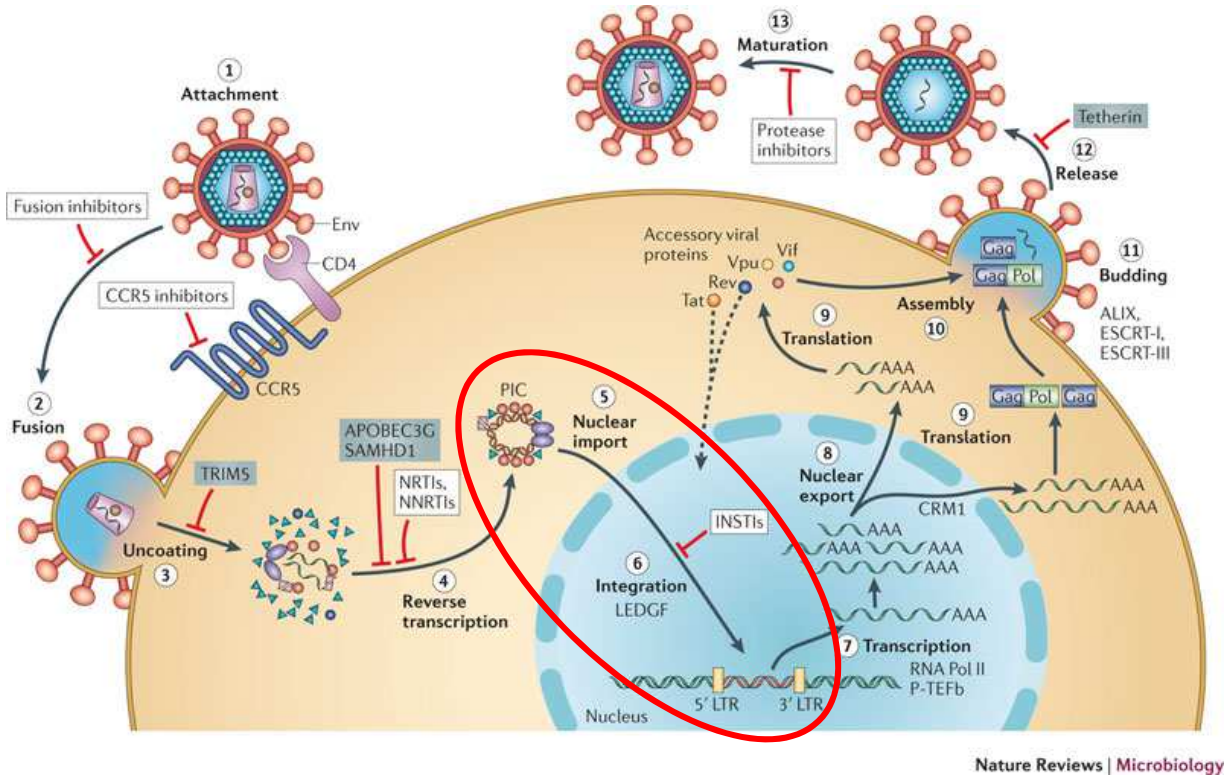


Adapted from J.M. Watts et al., 2009, Nature

Schematic diagram of HIV multiplication cycle

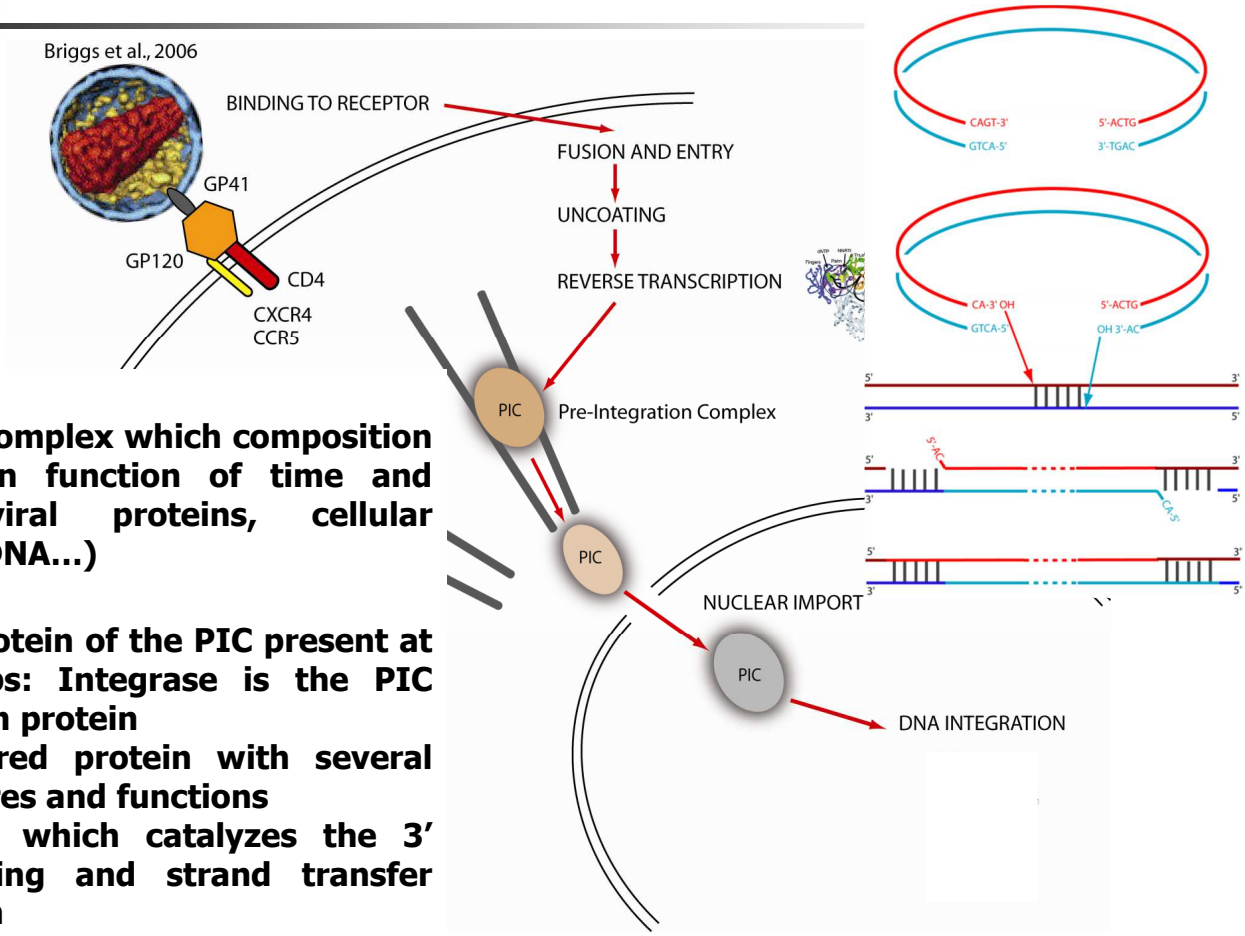


HIV multiplication cycle



Alan Engelman and Peter Cherepanov, Nature Review in Microbiology, 2012, 10, 279-289

HIV-1 pre-integration complex



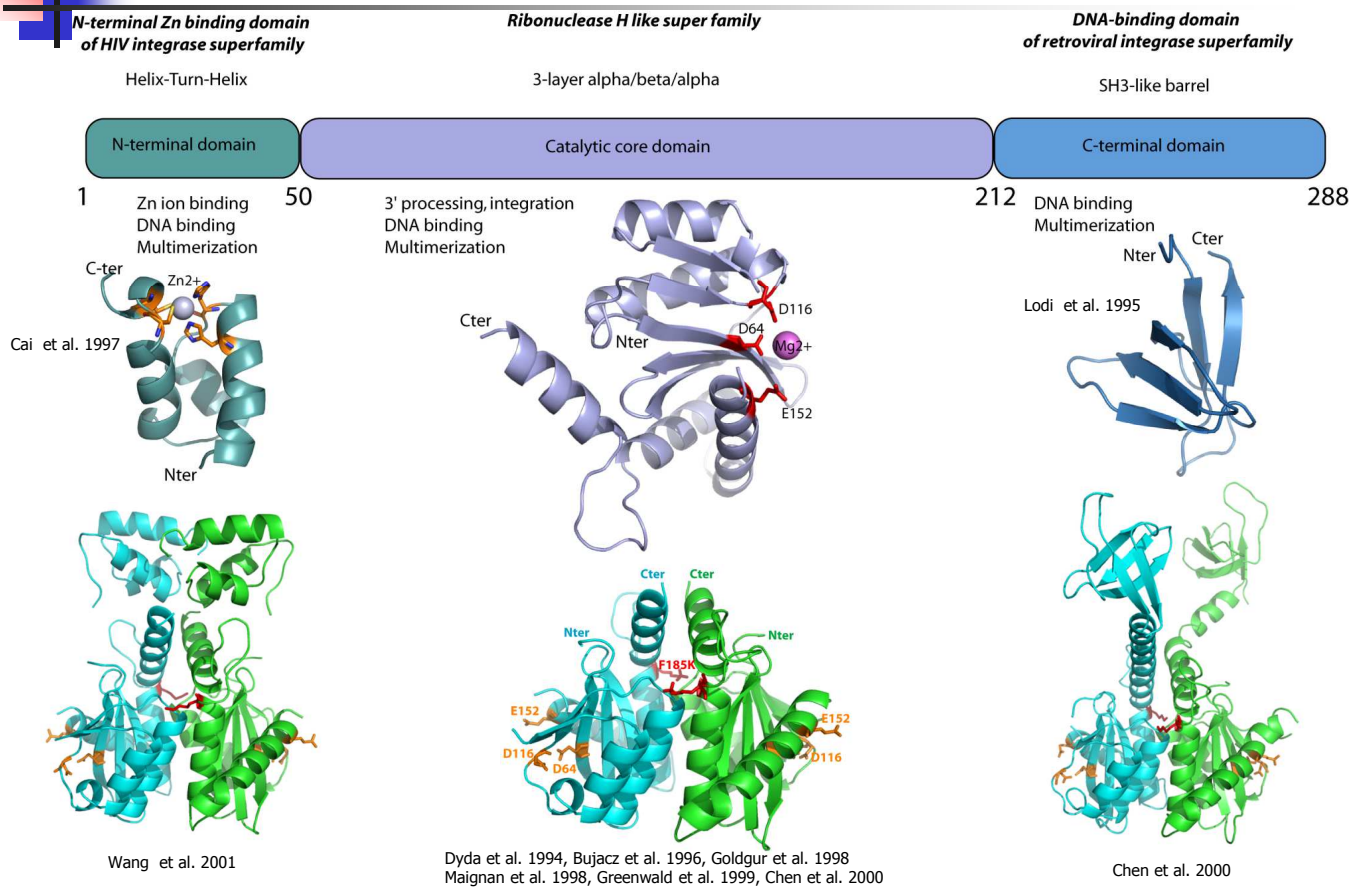
PIC:

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**
- **Disordered protein with several structures and functions**
- **Enzyme which catalyzes the 3' processing and strand transfer reaction**

HIV Integrase

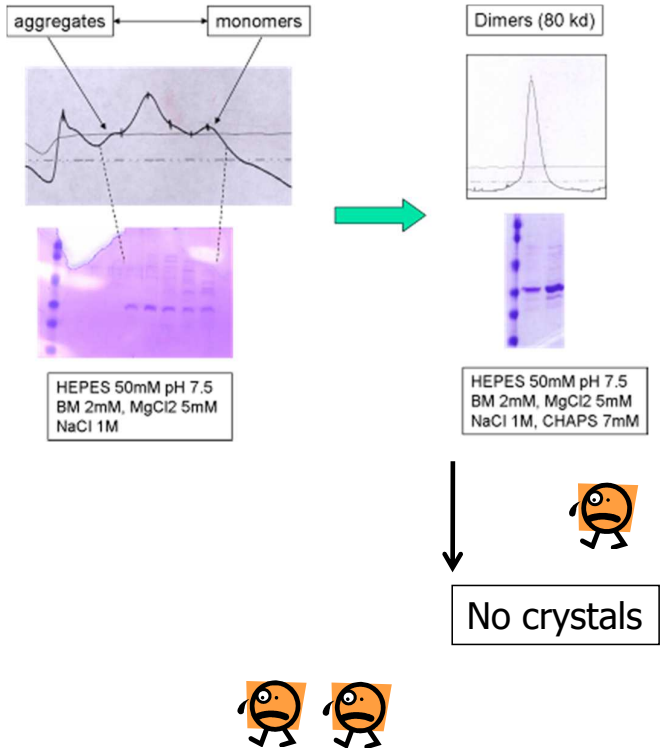


Organization of domains ? Mechanism of DNA integration ?

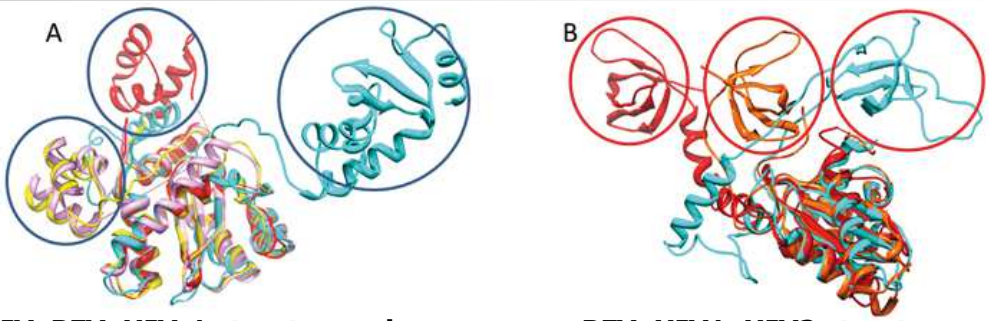
Full length Integrase wt : expression and solubilization

	37°		25°		18°	
	ET	EB	ET	EB	ET	EB
LB	+	-	+	+	+	+
LB/sucrose	+	-	+	+	++	++

	-	Glycerol 10%	Chaps 10mM	Triton 0.1%	Sucrose 20%
50mM NaCl	+/-	+/-	++	+/-	+/-
1M NaCl	+	++	+++	+++	++



HIV Integrase



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

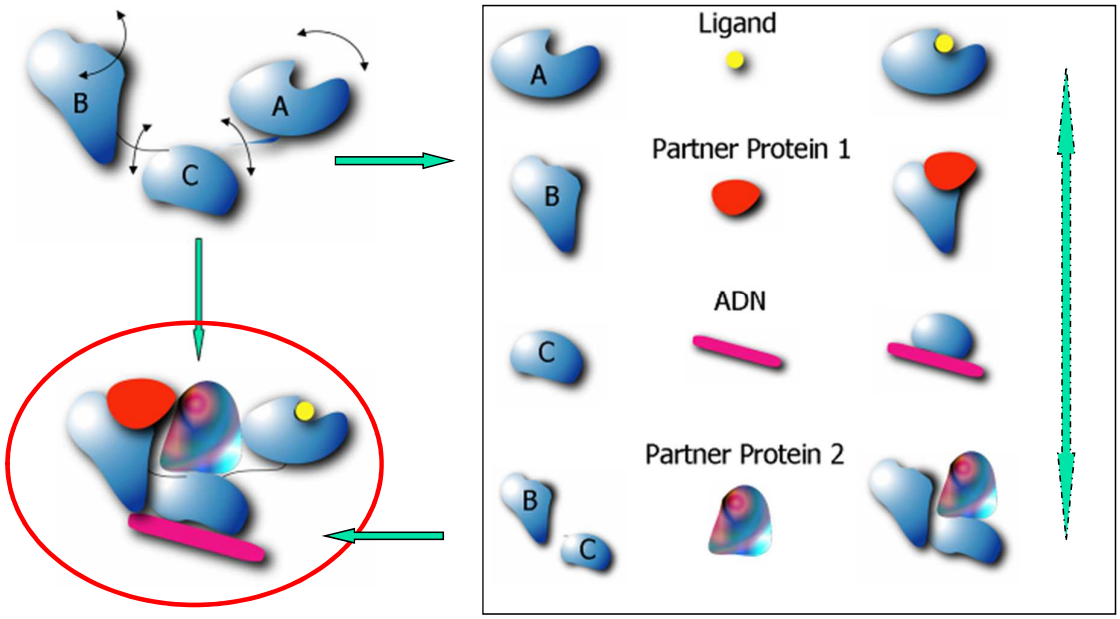
Structural and functional studies

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

Isolate protein with PTMs: Production in eukaryotic cells Specific modifications

Strategy

- Partial non-structuration of the protein (disordered regions)
- High inter domain mobility

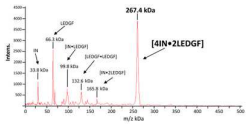


- Proteins domains, mutants, solubility fusions
- Stabilization by the interaction with interacting proteins, ligands, DNA.

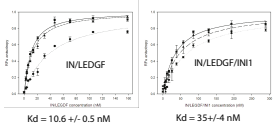
HIV PIC Structural and Functional studies

Cellular Cofactors

Viral cofactors
DNA, RT, CA,
Vpr, NC, Rev…

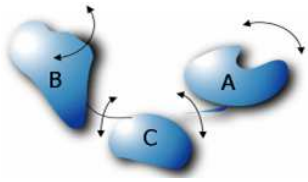


Mass spectrometry



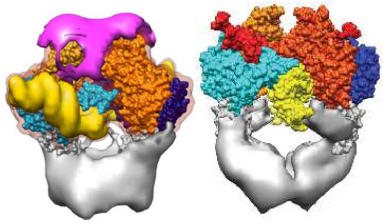
Functional validation in vitro

Structures (Xray, CryoEM)

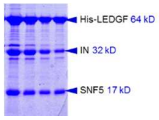


HIV integrase

Stable complexes



Maillot B. et al,(2013) PLoS ONE, e60734
Michel F. et al,(2009) EMBO J, 28, 980-991

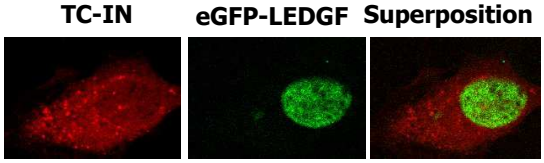


TRNSR2
VBP1
GCN5
P300
…

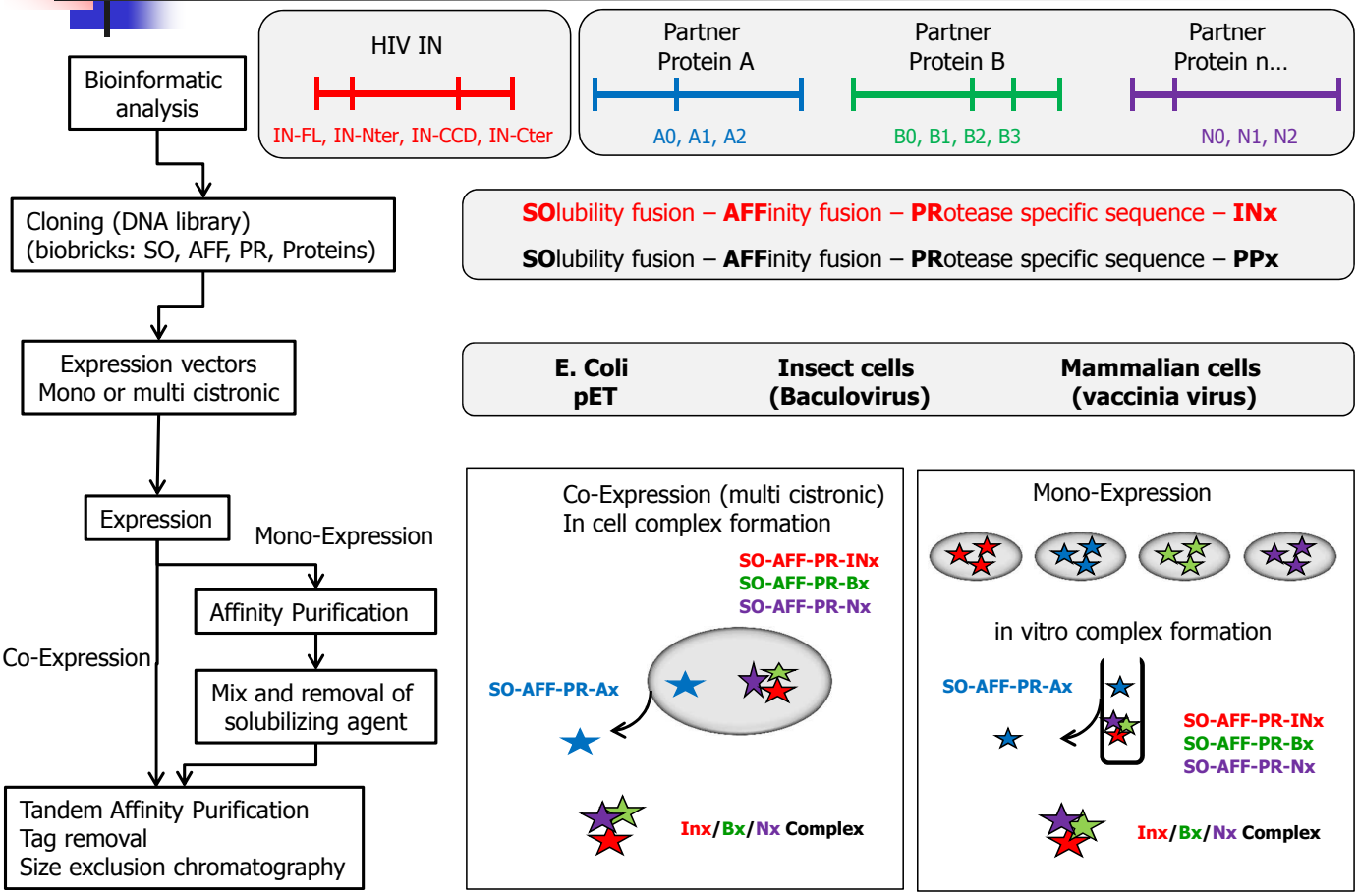
Post translational modifications

Small molecules

Functional validation in vivo
Complexes in the cellular context



From unstable protein to stable complexes: Pipeline procedure for stable complex characterization and production



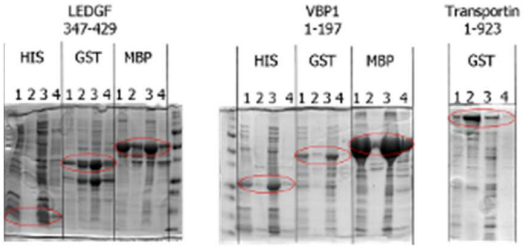
HIV-1 IN/LEDGF complex

Cloning, expression and solubility tests (E. Coli)

Protein	Fragment	Tag N_{term}	Protease site	Antibiotic	Expression	S_HS	S_LS	Notes
LEDGF	1-530	No	No	Chlo	++	++	Nd	
		No	Thrombin	Amp	++	+++		
		6xHis		Amp	+++	+++		
		6xHis		Zeocin	+++	+++		
		GST		Amp	+	+++		
		MBP		Amp	+	+++		
		6xHis-Nus		Amp	+	+++		
		Flag		Amp	Nd			
		No	Tev	Amp	Nd			
		6xHis		Amp	+++	++	++	
		GST		Amp	+	++	++	
		No	Protease 3C	Amp	Nd			
		6xHis		Amp	+++	++	++	
		GST		Amp	+	++	++	
		6xHis		Spec	+++	++	++	Bicistronic (IN)
	347-429	No	No	Chlo				
		6xHis	Protease 3C	Amp	+++	++	++	
		GST		Amp	+++	+++	+++	
		MBP		Amp	+++	+++	++	
		6xHis		Spec				Bicistronic (IN)
	347-442	No	No	Chlo	Nd			
		6xHis	Tev	Amp	Nd			
		GST		Amp	Nd			
		6xHis-MBP		Amp	Nd			
		6xHis	Protease 3C	Amp	Nd			
		GST		Amp	Nd			
		6xHis-MBP		Amp	Nd			
	347-471	No	No	Chlo	Nd			
		6xHis	Tev	Amp	Nd			
		GST		Amp	Nd			
		6xHis-MBP		Amp	Nd			
		6xHis	Protease 3C	Amp	Nd			
		GST		Amp	Nd			
		6xHis-MBP		Amp	Nd			

Protein	Fragment	Tag N_{term}	Protease site	Antibiotic	Expression	Solubility	Notes
Integrase	1-288	No	No	Chlo	+	+	Only in co-expression
		No	Thrombin	Amp	+	+	
		6xHis		Amp	++++	+++	pET15b
		6xHis		Amp	+	++	
		6xHis		Zeocin	++	++	
		6xHis		Kana	++	++	
		GST		Amp	++	+++	
		MBP		Amp	+++	+++	Affinity Pb
		6xHis-Nus		Amp	++	+++	
		Flag		Amp	Nd		
		No	Tev	Amp	Nd		
		6xHis		Amp	Nd		
		GST		Amp	-		Only GST exp
		No	Protease 3C	Amp	Nd		
		6xHis		Amp	++	+++	
		GST		Amp	+++	+++	
		6xHis		Chlo	+	+	Bicistronic (LEDGF)

Protein	Fragment	Tag N_{term}	Protease site	Antibiotic	Expression	Solubility HS	Solubility LS	Notes
VBP1	1-197	6xHis	P3C	Amp	++	+++	Nd	
		GST			+++	+++		
		MBP			+++	+++		
		6xHis-MBP			+++	+++		
		6xHis	Tev		+++	+++		
		GST			+++	+++		
		6xHis-MBP			+++	+++		
		6xHis	P3C	spec	Nd	Nd		Bi-cistronic
		0		Chlo	Nd	Nd		
SNF5	1-385	6xHis	Tev	Amp	-	-		
		GST			+++	+++		
		6xHis-MBP			+++	+++		
		6xHis	P3C		-	-		
		GST			+++	+++		
		6xHis-MBP			+++	+++		
		0		Chlo	Nd	Nd		
Transportin	1-923	GST	Thrombin	Amp	+++	++	++	pGEX



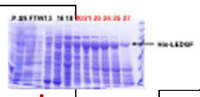
	37°		25°		18°	
	ET	EB	ET	EB	ET	EB
LB	+	-	+	+	+	+
LB/sucrose	+	-	+	+	++	++

	-	Glycerol 10%	Chaps 10mM	Triton 0.1%	Sucrose 20%
50mM NaCl	+/-	+/-	++	+/-	+/-
1M NaCl	+	++	+++	+++	++

IN/LEDGF : complex formation and purification: E. Coli

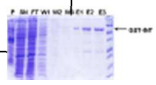
Purification GST-P3C-INT and HIS-Thr-LEDGF

Purification His-Thr-LEDGF



High salt, detergent

Purification GST-P3C-INT



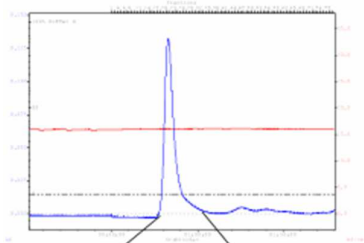
Complex formation by dialysis

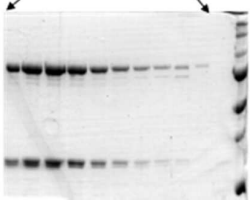
GST Affinity

P3C cut

GST Affinity

Gel filtration G200





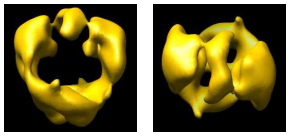
Functional and biochemical characterization

HTS Screening of inhibitors

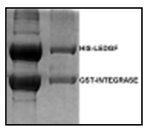
Crystallization Assays

SAXS, SANS studies

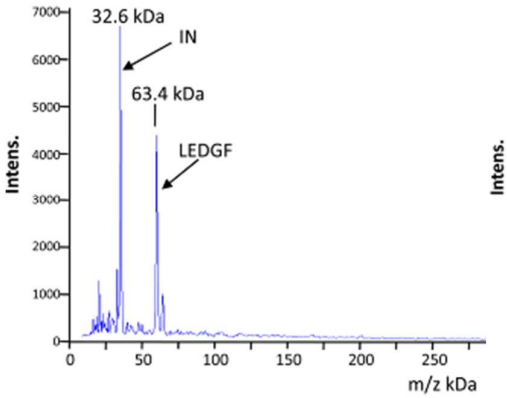
EM studies



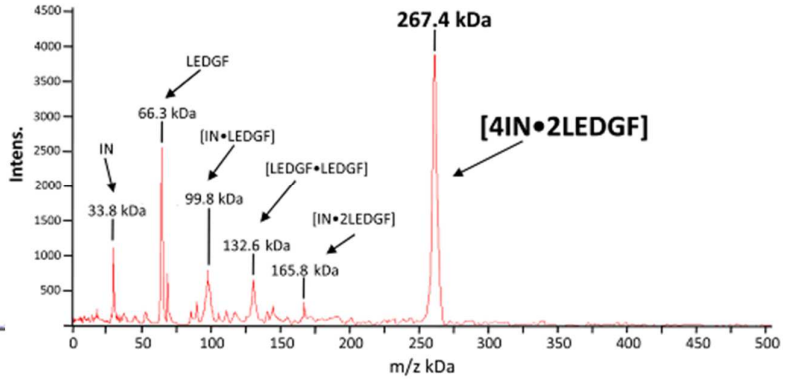
Yield: 5 mg of complex (INT 5L, LEDGF 6L)



IN/LEDGF : characterization by High Mass MALDI-TOFF



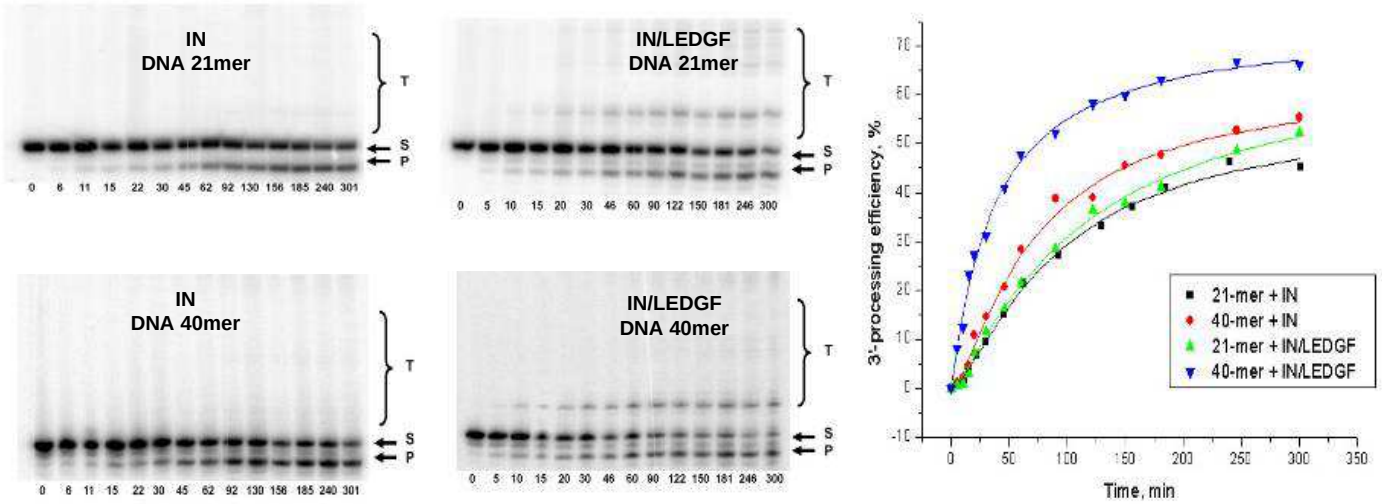
Absence of cross linking agents



Presence of cross linking agents

The cross-linking reactions were realised using a solution containing different cross-linkers specific for amino and sulfhydryl groups. The cross linking reactions were performed using a reagent composed of iodoacetec acid N-hydoxysuccinimide ester, Octaneodic acid di-N-hydroxysuccinimide ester and ethylene glycol bis-succinimidylsuccinate. (K200 MALDI MS analysis Kit, CovalX AG, Zürich, Switzerland).

IN/LEDGF : Functional characterization

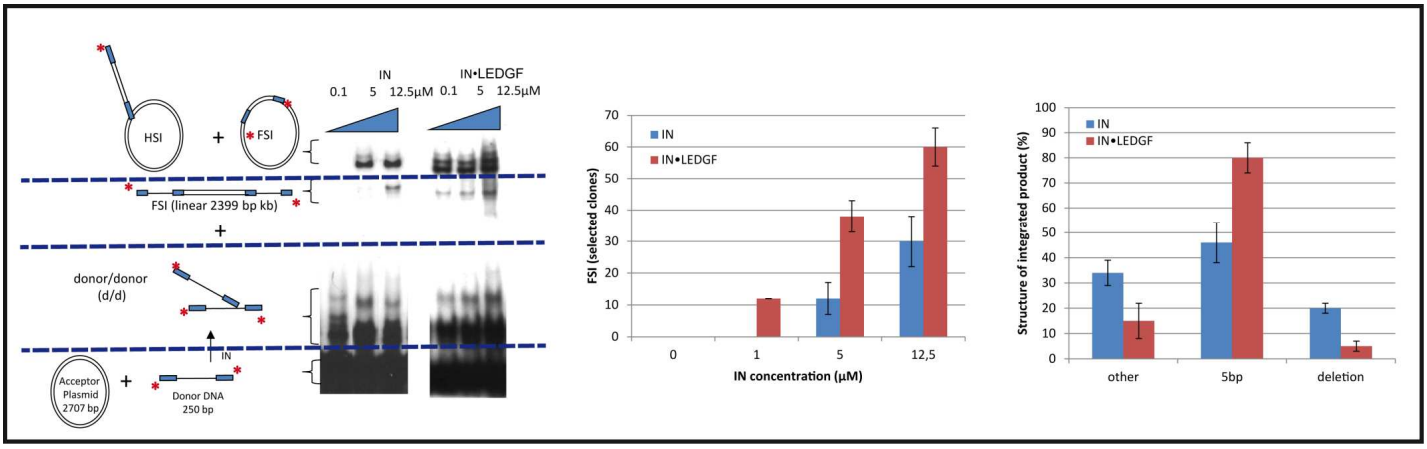


DNA: 21 or 40-mer duplex that mimic the HIV-1 U5 viral DNA end

5′-**GACTACGGTTCAAGTCAGC**GTGTGGAAAATCTCTAGCAGT-3′
3′-**CTGATGCCAAGTTCAGTCG**CACACCTTTTAGAGATCGTCA-5′

LEGDF stimulates the 3′ processing activity of IN with a 40-mer DNA.
In the presence of LEGDF, the strand transfer efficiency is strongly enhanced for both the 21- and the 40-mer DNA.

IN/LEDGF : Functional characterization



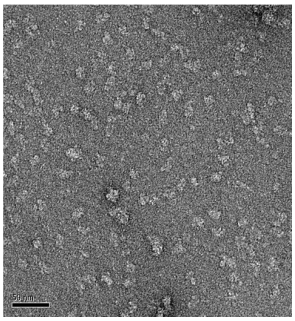
The global integration efficiency is higher for the IN/LEDGF complex than for isolated IN molecules. Specific cloning and quantification of the circular FSI products attested that the IN/LEDGF complex catalyzes more concerted integration events than isolated IN molecules. The integration reaction catalyzed by the IN/LEDGF complex is closer to the expected physiological reaction than IN alone (5bp staggered cuts of the target DNA).

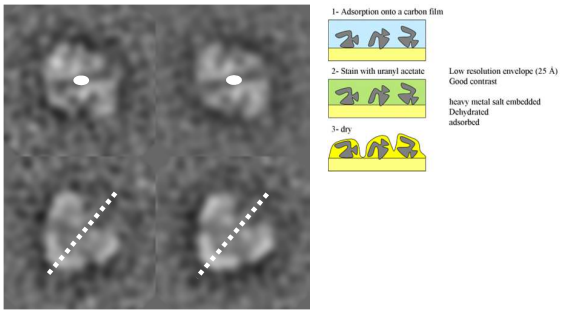
3D reconstruction of the complex IN/LEDGF

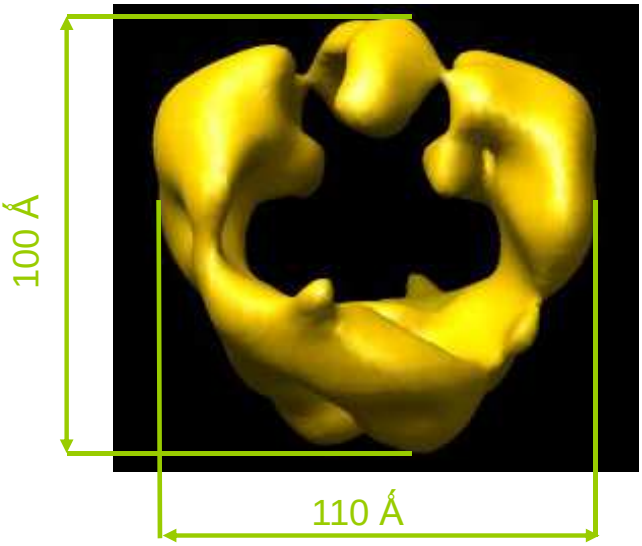
Round particles about 12 nm in diameter

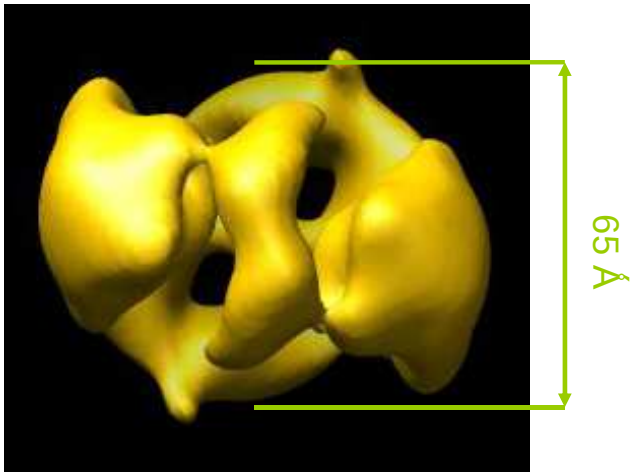
4000 NS images were collected

First structure
Resolution: 30 Å

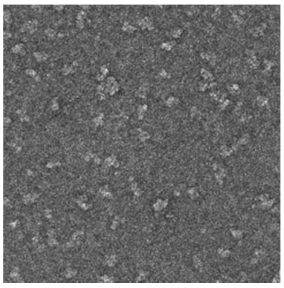




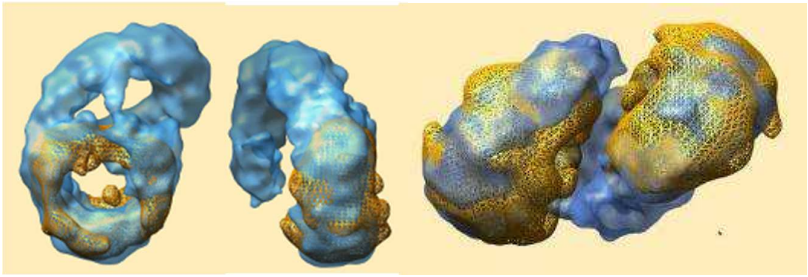




Negatively stained EM of GST-IN/LEDGF complexes

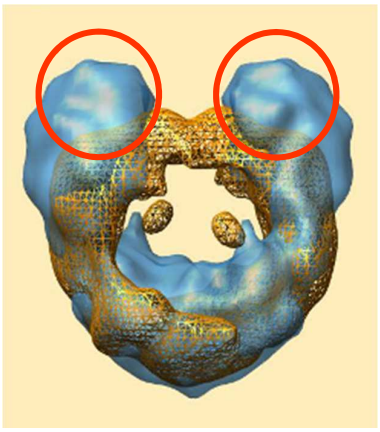


6134 images collected



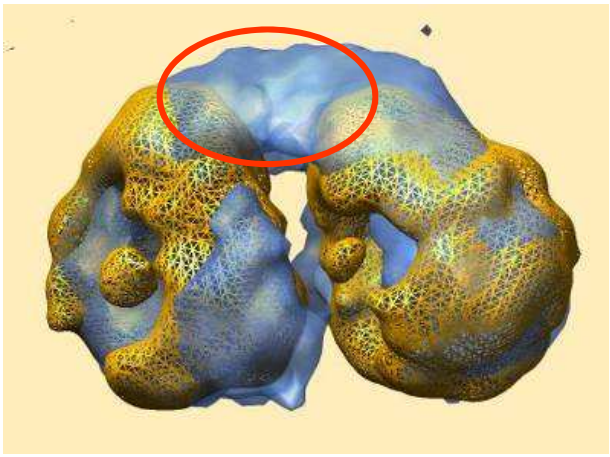
The original form of the In-LEGDF complex is clearly visible

The two fold axis is clearly visible without imposing any symmetry

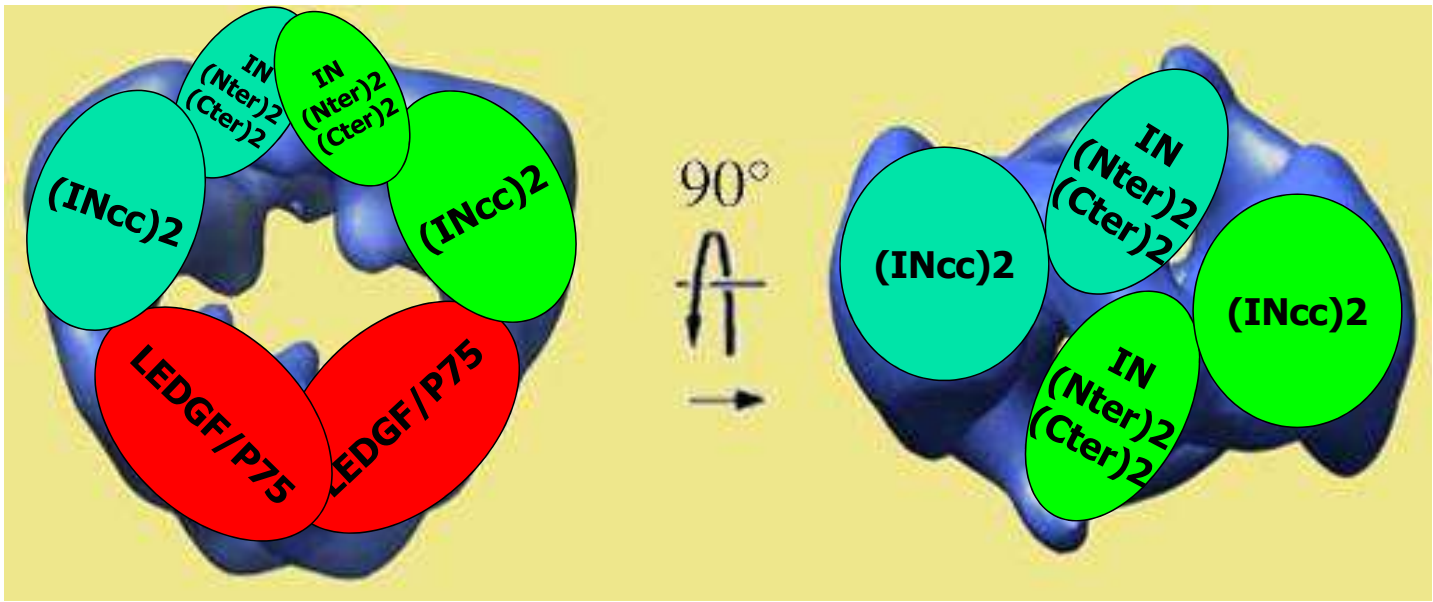


GST induced dimerization

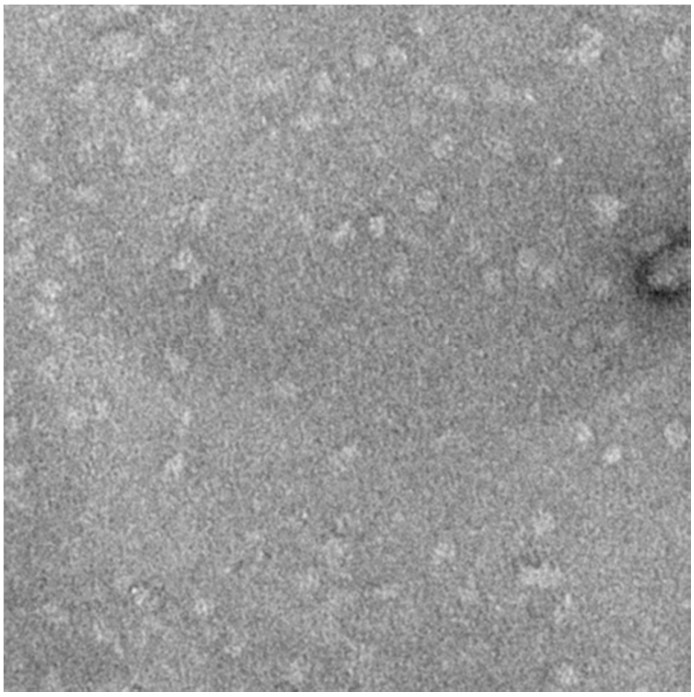
Unambiguous positioning of integrase



Possible localization of proteins

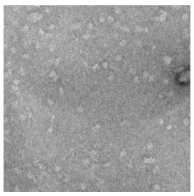


IN/LEDGF: Cryo-EM

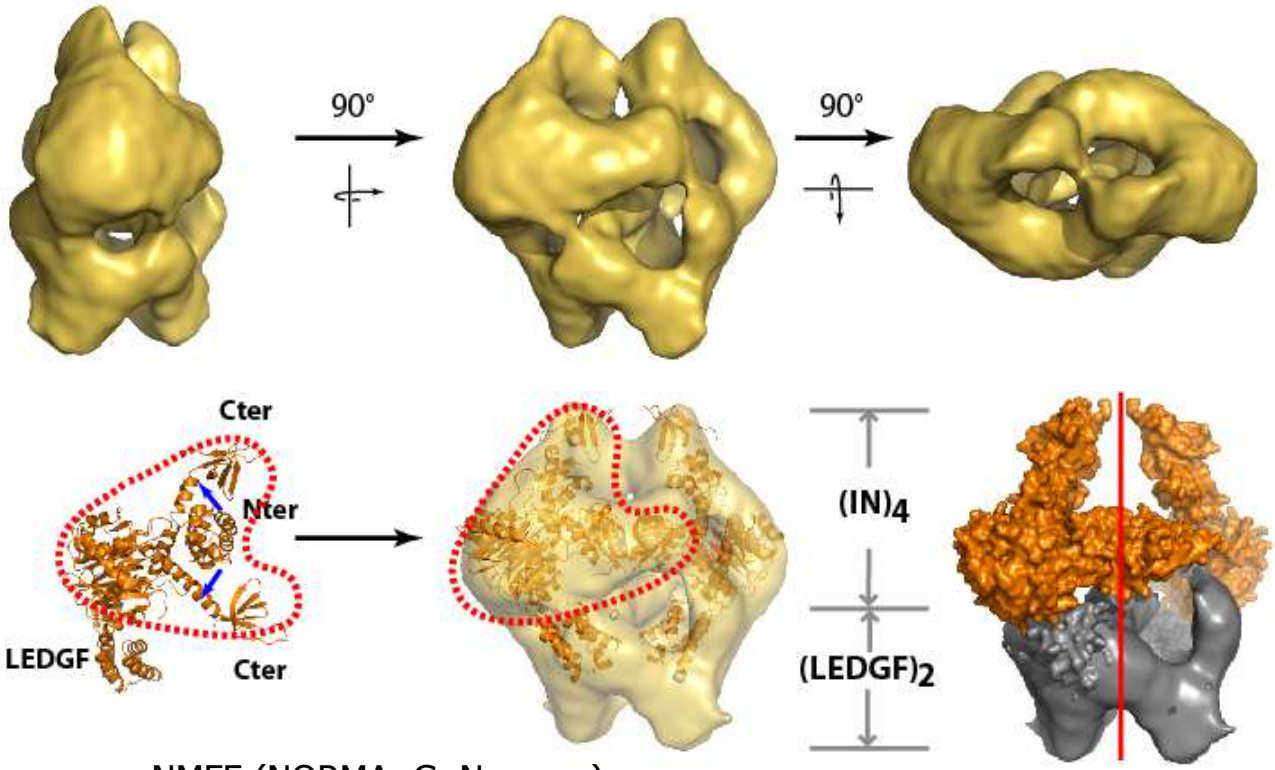


11441 images were collected

IN/LEDGF: CryoEM, model at 14 Å

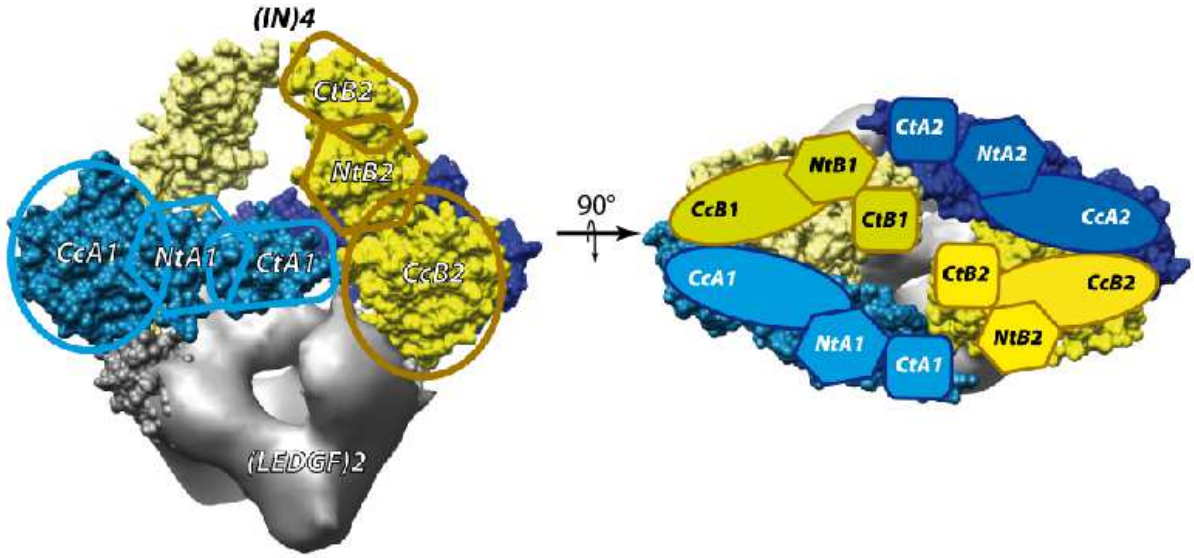


11441 Images collected



NMFF (NORMA, G. Navazza)

IN/LEDGF: Domain organization

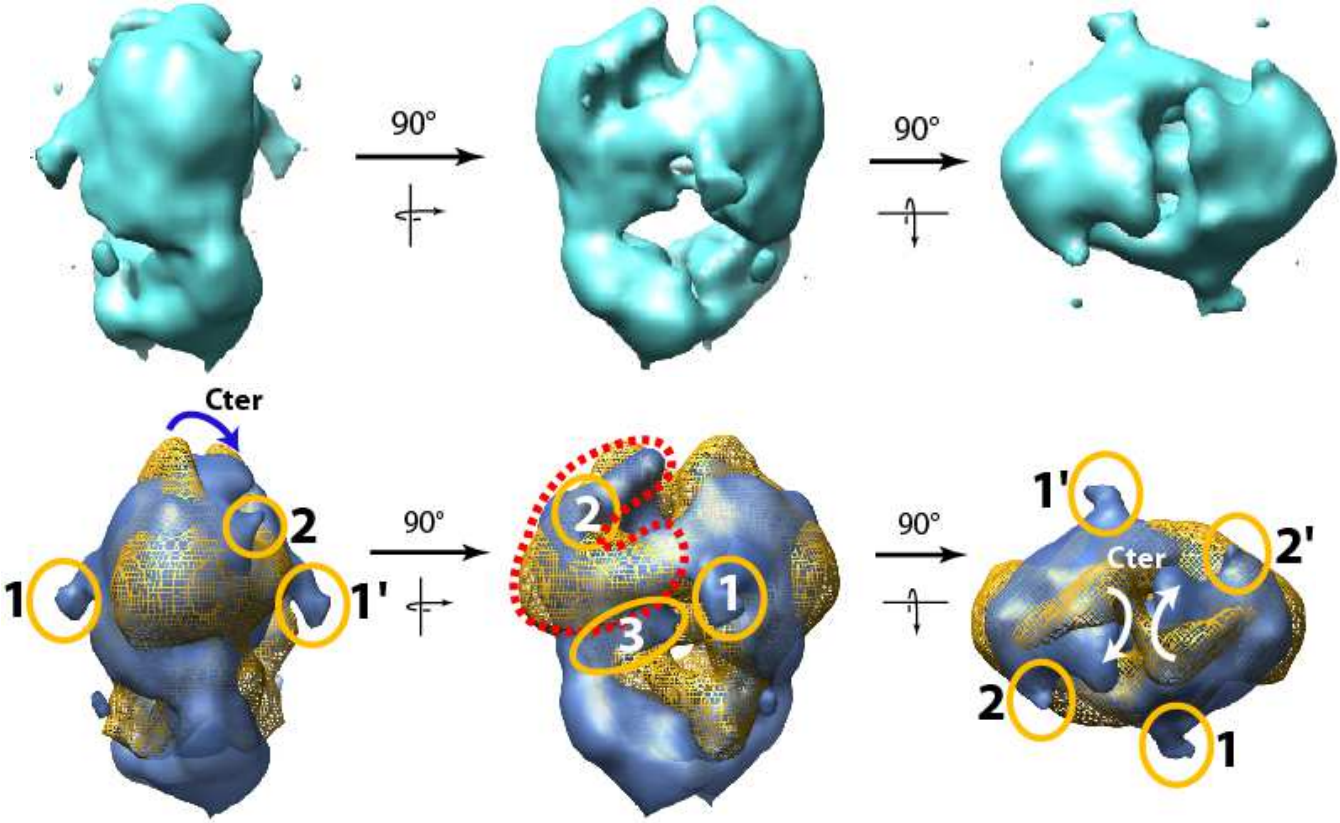


IN-LEDGF complex contains 4 IN molecules (A1, A2, B1 and B2) organized in two IN dimers

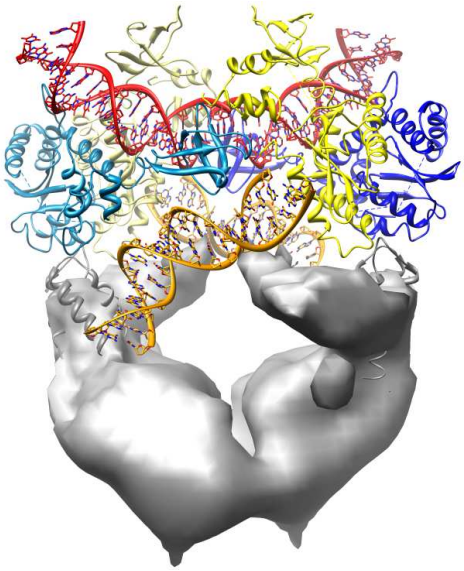
Each IN monomer within the IN dimer has a different conformation → the IN dimer is asymmetric

Each IN molecule has a distinct function within the dimer.

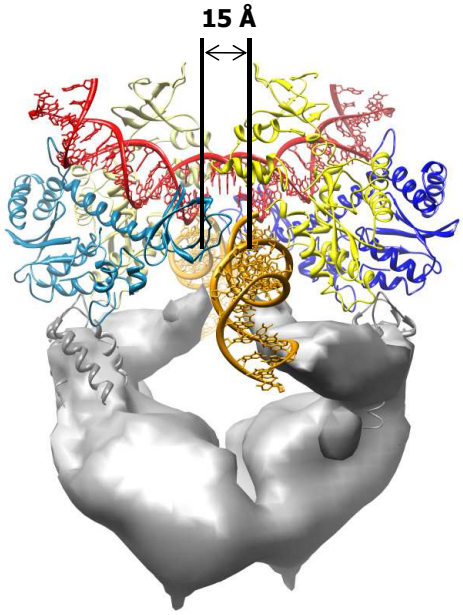
IN/LEDGF/DNA-21mer: CryoEM, model at 14 Å



Models of IN/LEDGF/DNA complex



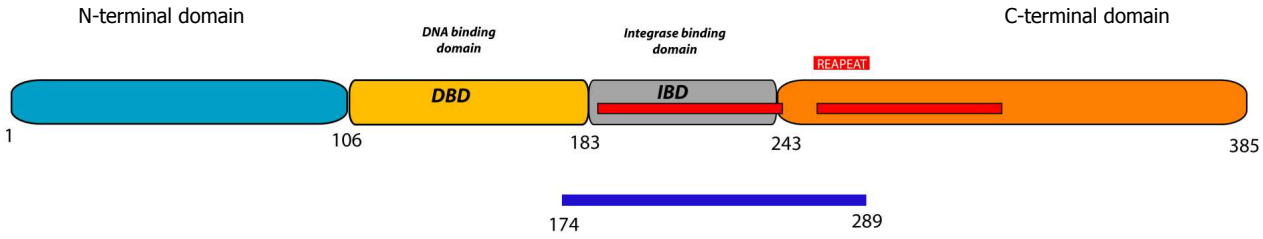
3′ Processing



Integration

HIV-1 IN/LEDGF/INI1 complex

IN interact with INI1/SNF5

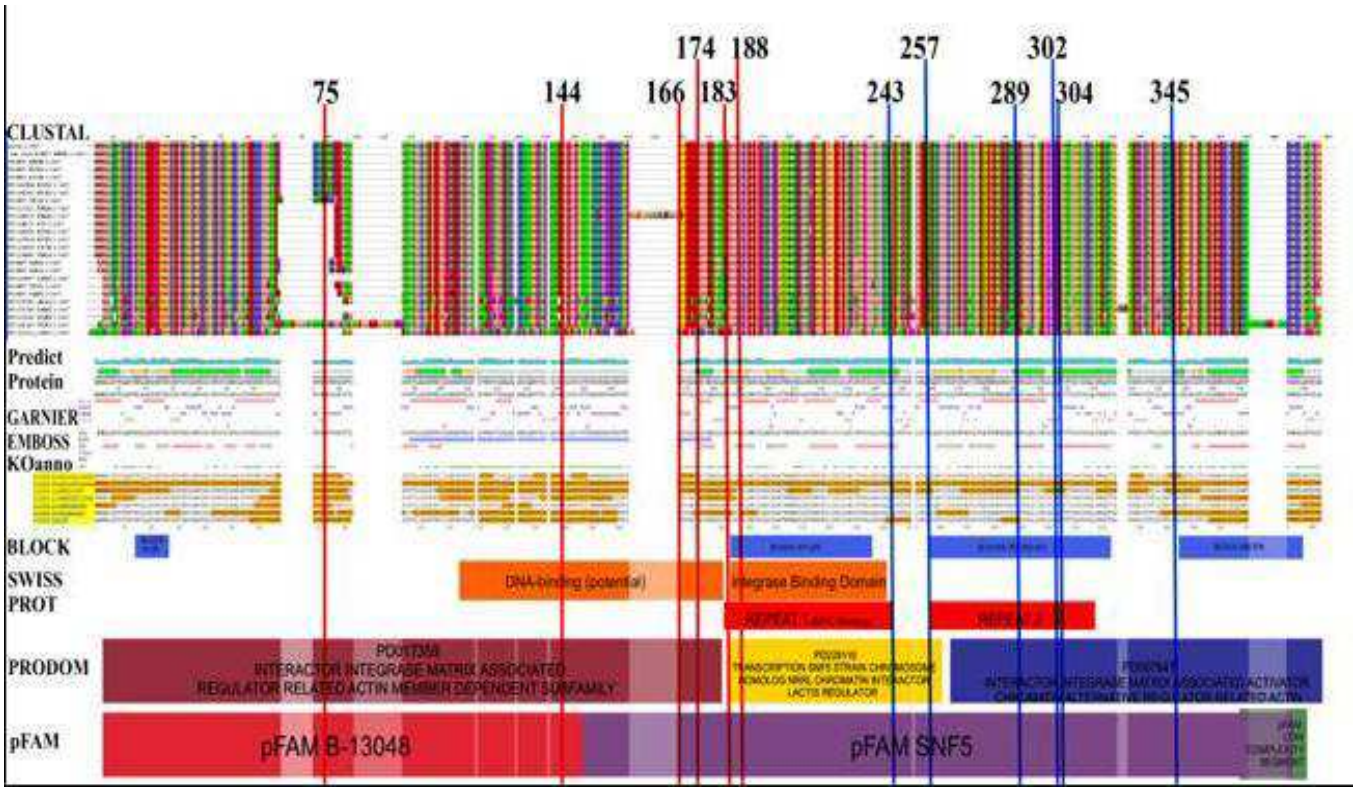


SNF5/Ini1, a subunit of the SWI/SNF chromatin remodeling complex, is the first cofactor identified to interact with IN.

SNF5/Ini1 is one of the core subunits of the ATP-dependent chromatin remodeling complex SWI/SNF that regulates expression of numerous eukaryotic genes by altering DNA/histone interactions

It has been postulated that SNF5/Ini1 could target PICs to regions of the genome that are enriched for the SWI/SNF complex

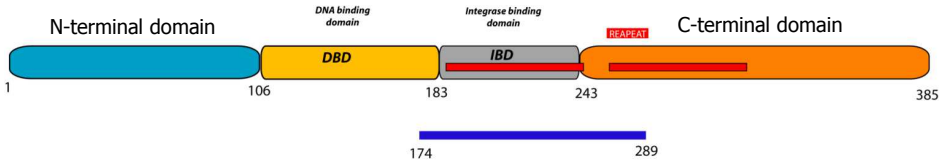
INI1/SNF5: bioinformatic analysis



INI1: Cloning, expression and solubility tests (E. Coli)

	NaCl				
pH7,5	150mM	500mM	1M	2M	2,5M
0	-	-	-	-	-
CHAPS 7mM	-	-	-	-	-
CHAPS 10mM	-	-	-	-	+
CHAPS 20mM		-	-	+	++
Z[3-12] 4mM		+++			

IN/LEDGF/INI1 : complex formation and purification



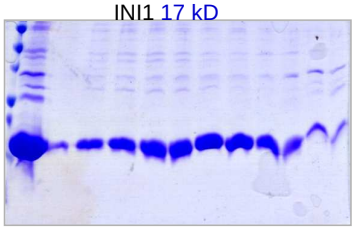
HIS-INI1(174-289) → His Affinity → Gel filtration G200

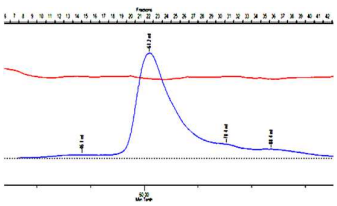
+ IN/LEDGF

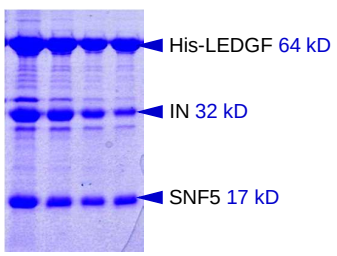
High salt detergent

Gel filtration G200

- Functional and biochemical characterization
- HTS Screening of inhibitors
- Crystallization Assays
- SAXS studies
- EM studies



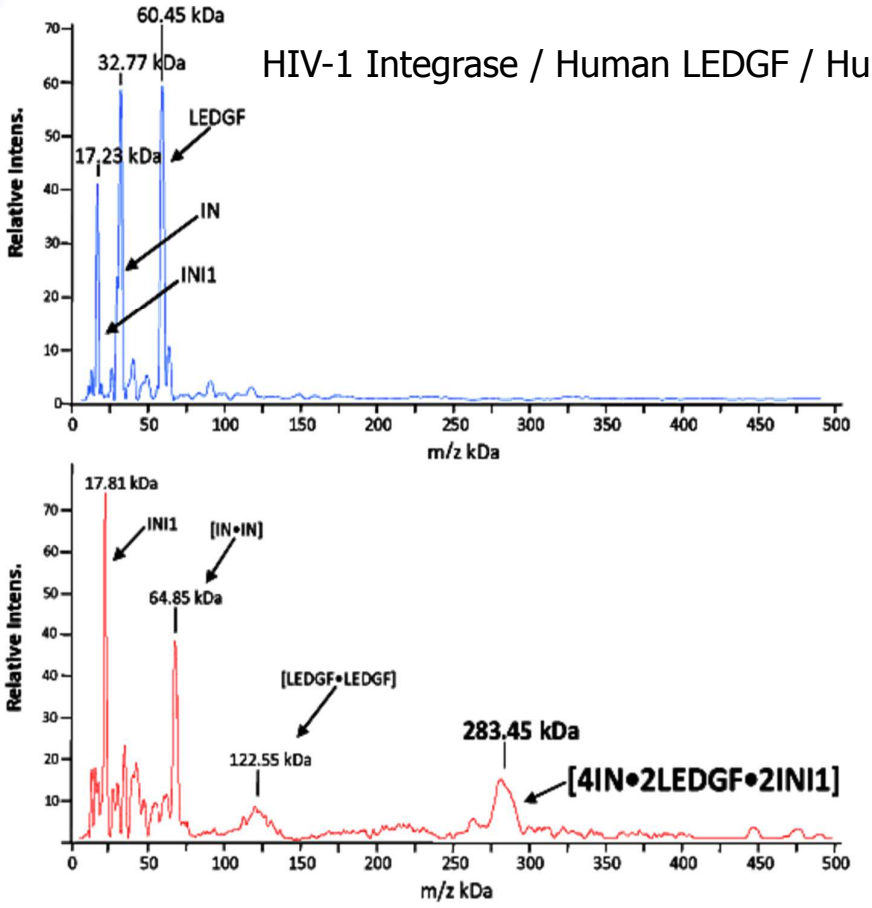




Yield: 2.5 mg of complex (INT 0.5L, LEDGF 0.5L, SNF5 1.0L)

Protein complexes analysis : High Mass MALDI-ToF

HIV-1 Integrase / Human LEDGF / Human INI1 (173-290)



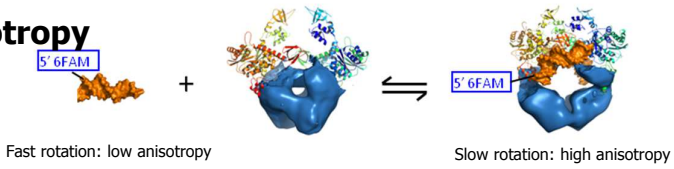
IN/LEDGF and IN/LEDGF/INI1: Functional characterization

Viral DNA Binding assay by fluorescence anisotropy

DNA: 40-mer duplex that mimic the HIV-1 U5 viral DNA end

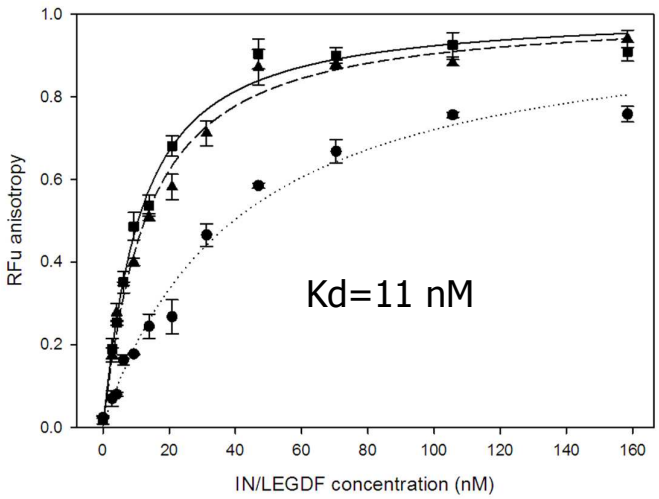
5′-GACTACGGTTCAAGTCAGCGTGTGGAAAATCTCTAGCAGT-3′

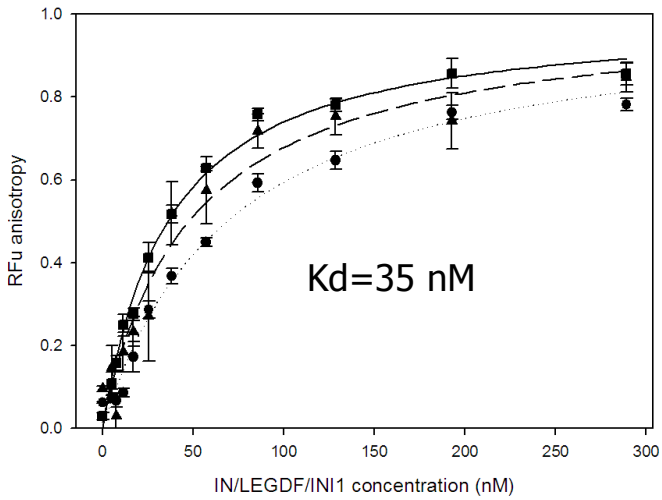
3′-CTGATGCCAAGTTCAGTCGCACACCTTTTAGAGATCGTCA-5′



Fast rotation: low anisotropy

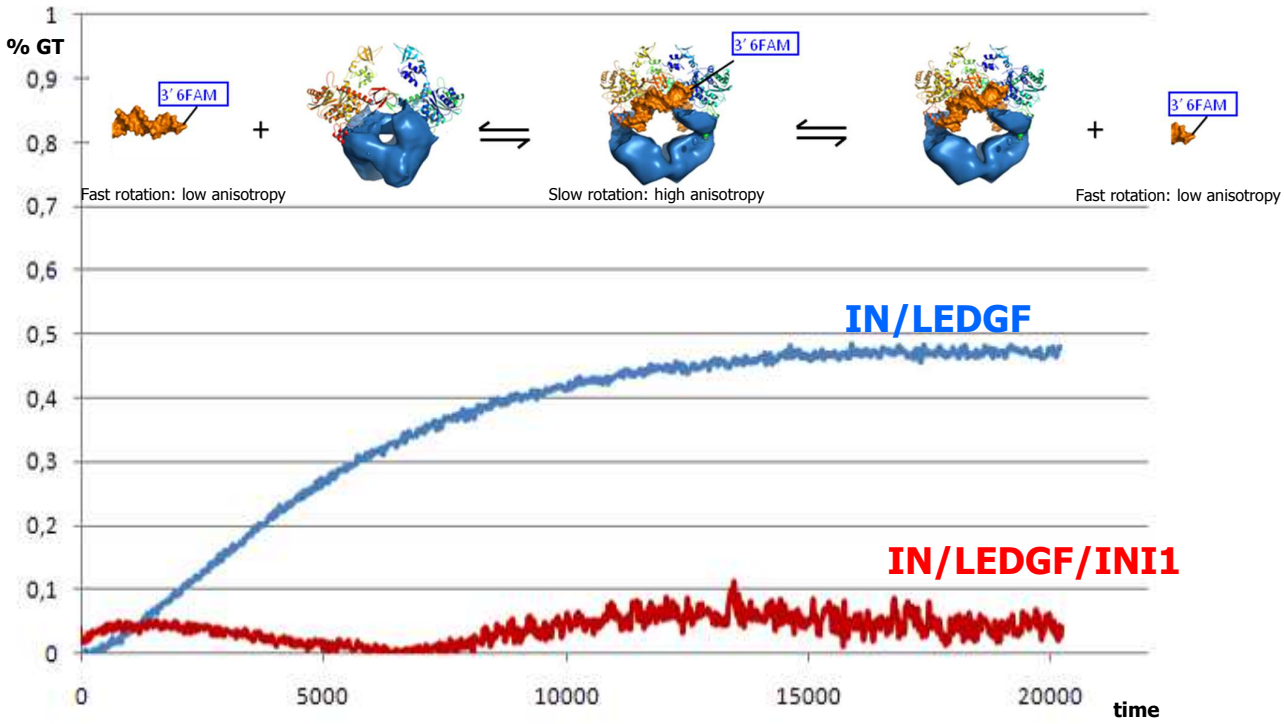
Slow rotation: high anisotropy





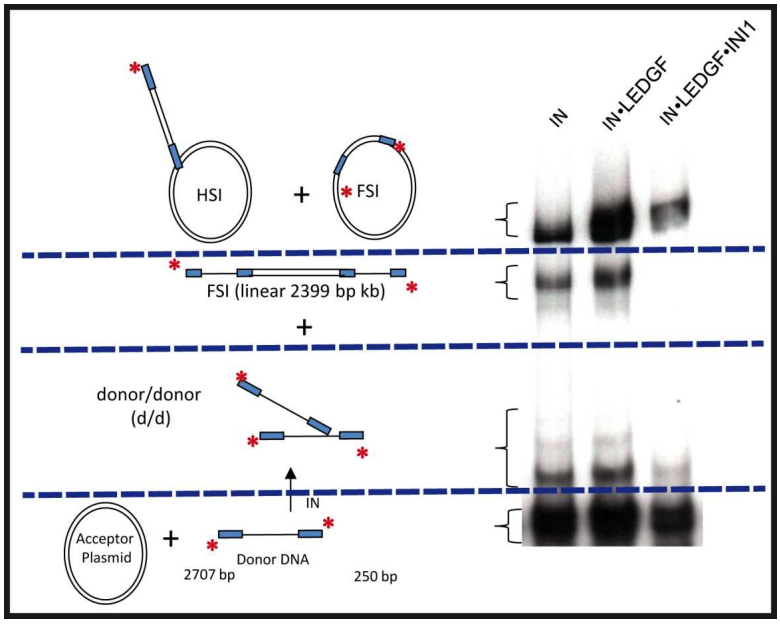
IN/LEDGF and IN/LEDGF/INI1: Functional characterization

The 3' Processing assay by fluorescence anisotropy



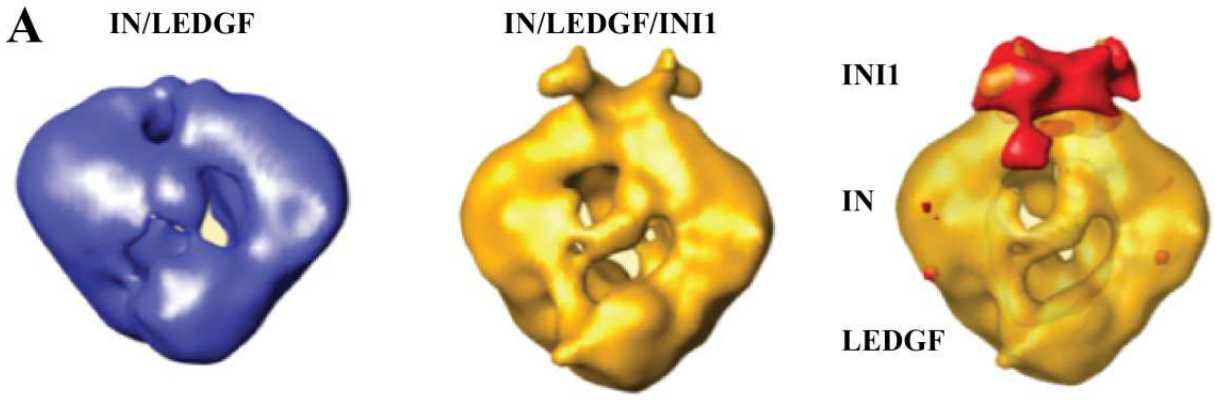
INI1 inhibits the *3' processing* activity of IN/LEDGF

IN/LEDGF and IN/LEDGF/INI1: Functional characterization



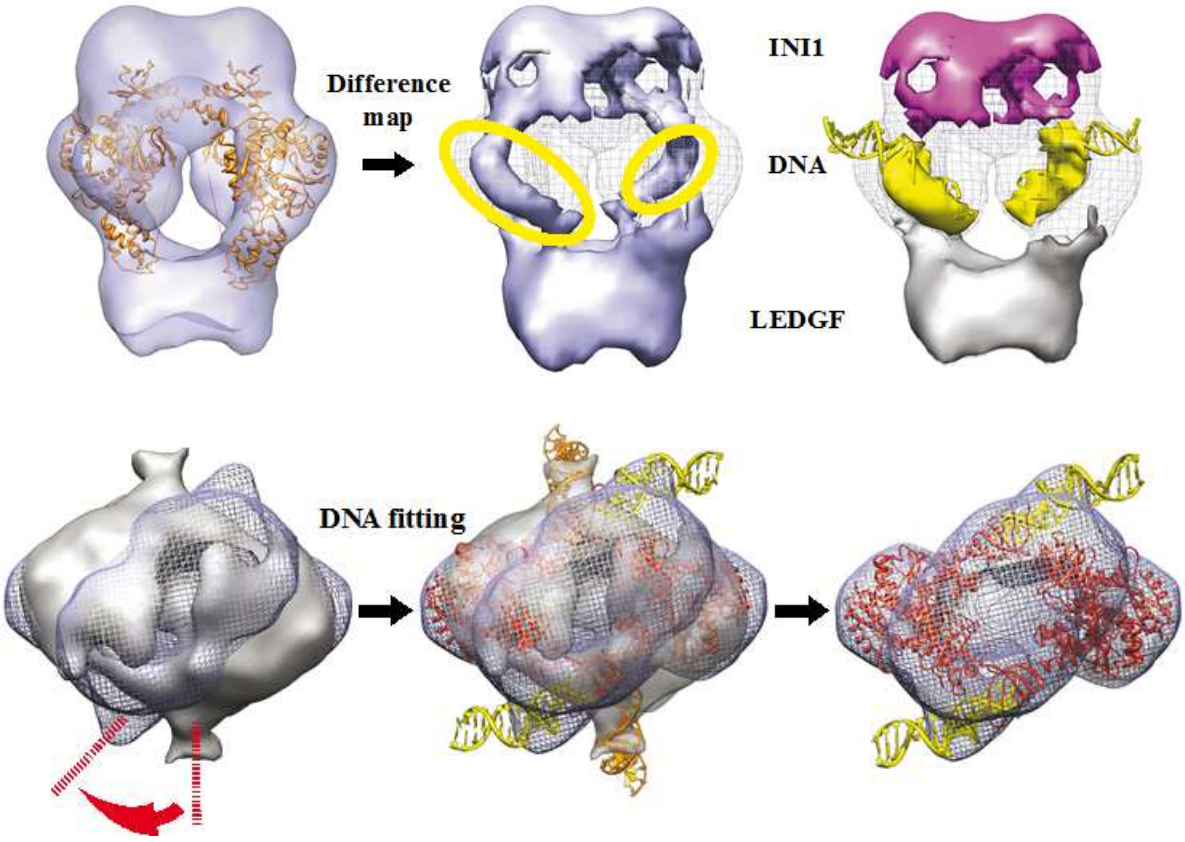
In the presence of INI1, integration occurs with reduced kinetics compared to IN alone or to the IN/LEDGF complex with strongly reduced by-products formation

Negative stained EM: 30 Å structures

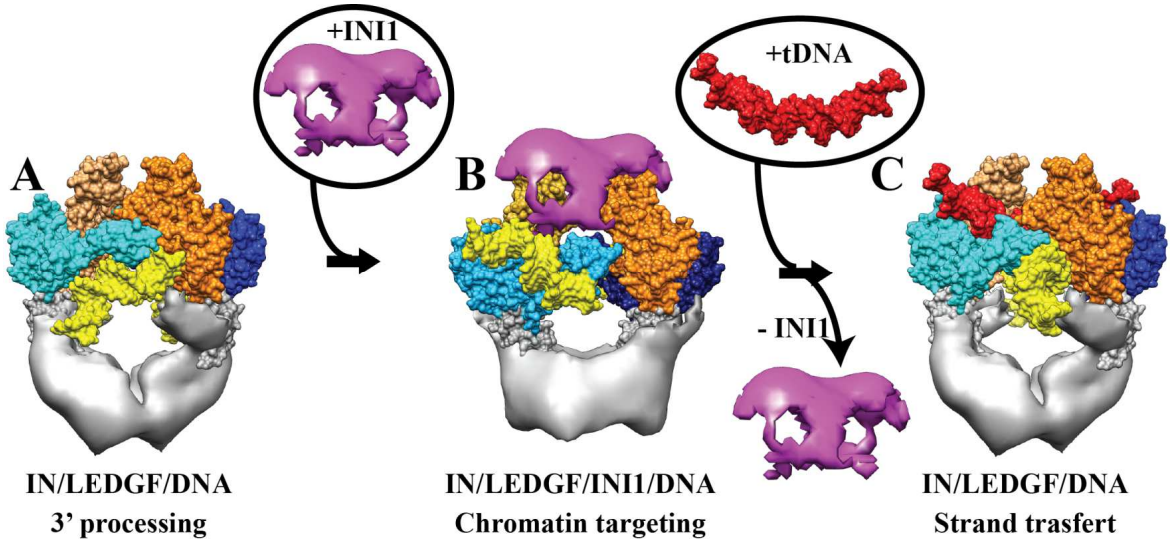


INI1 localization

IN/LEDGF/INI1/DNA: Cryo-EM structure

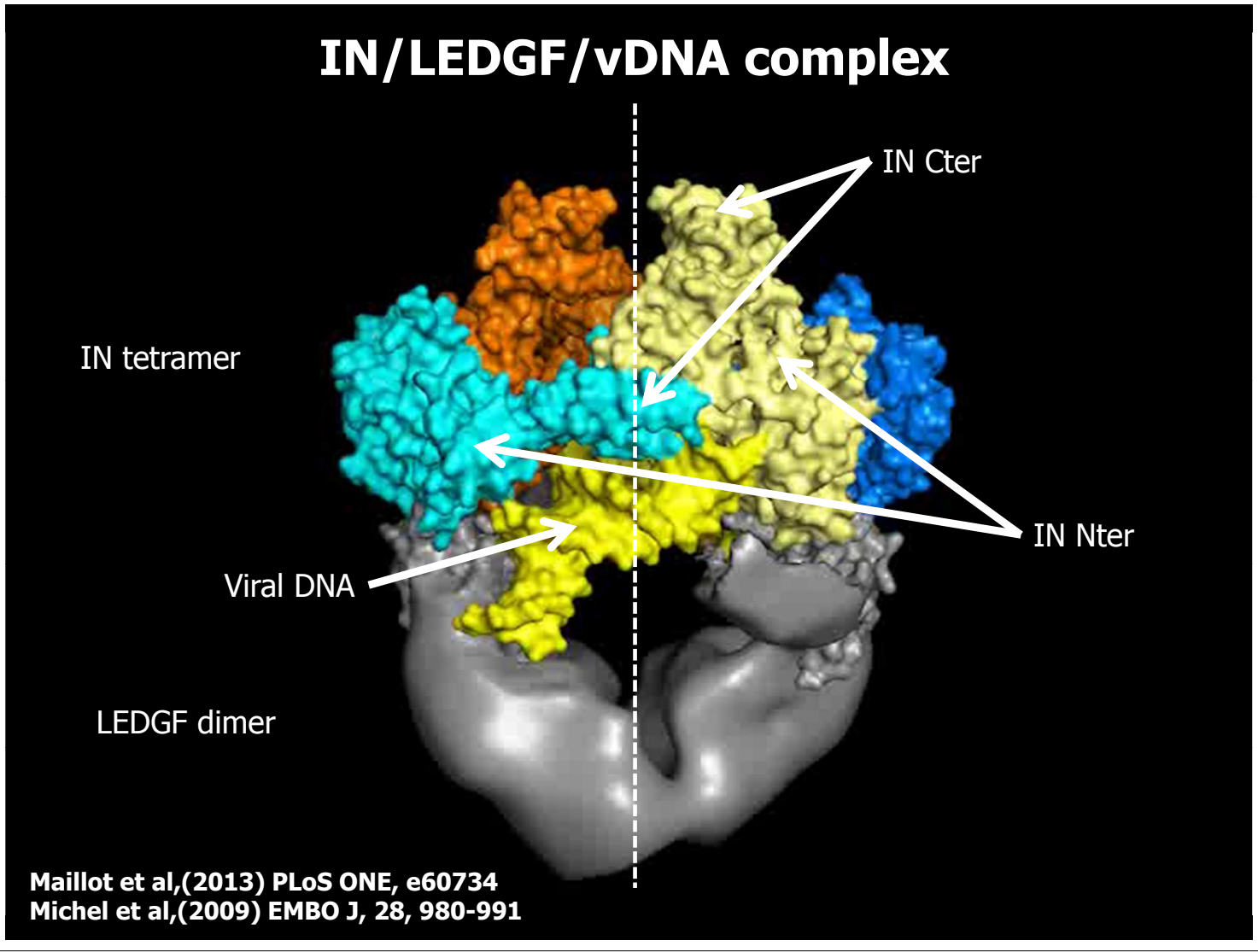


Function of INI1 in HIV-1 infection



Benoit Maillot, Nicolas Lévy, Sylvia Eiler, Corinne Crucifix, et al., (2013), Structural and functional role of INI1 and LEDGF in the HIV-1 preintegration complex, PlosOne, In Press

Michel, F., Crucifix, C., Granger, F., et al., (2009). Structural basis for HIV-1 DNA integration in the human genome, role of the LEDGF/P75 cofactor. EMBO J., 28, 980-991

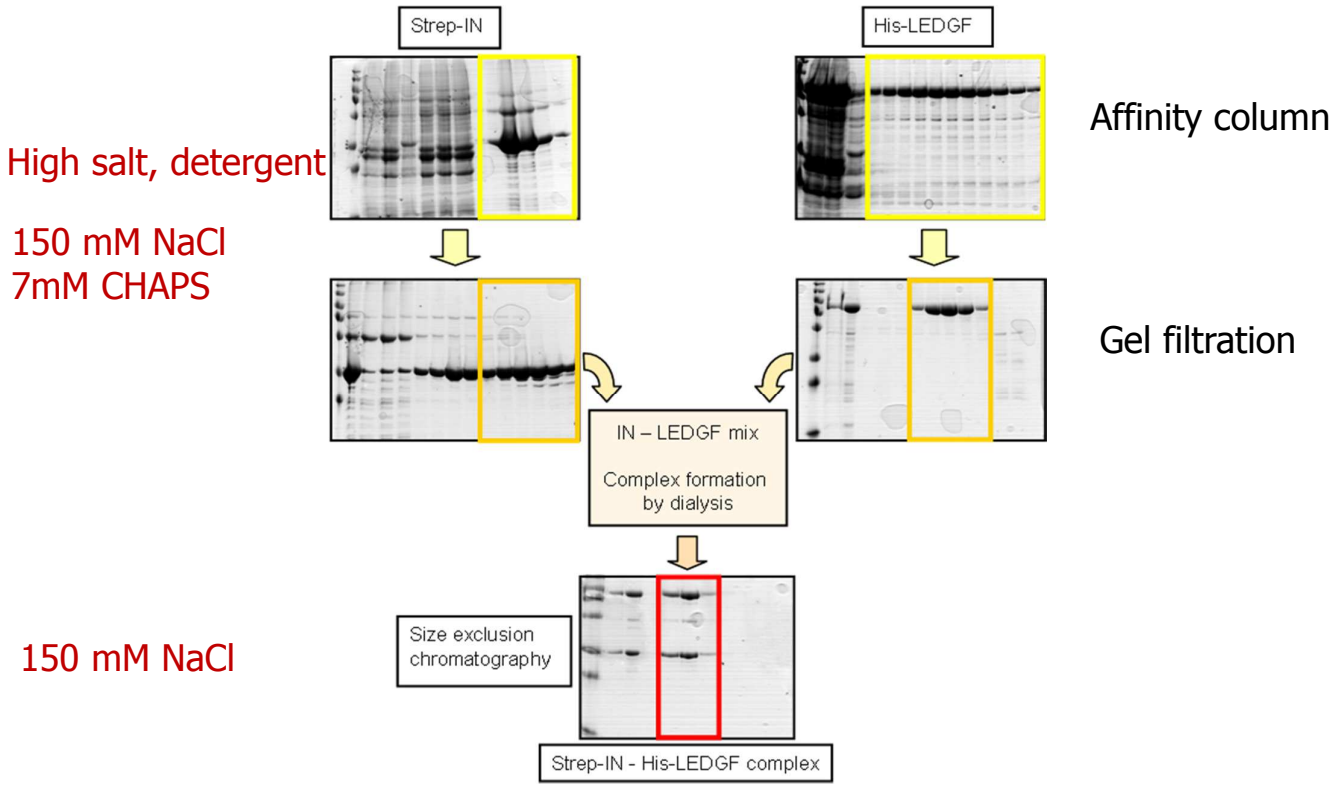


Integrase stability and solubility

Protein production in eukaryotic cells
Insect and Mammalian cells

IN/LEDGF : complex formation and purification (Insect cells)

Expression vector: Baculovirus



IN/LEDGF in mammalian cells (vaccinia virus)

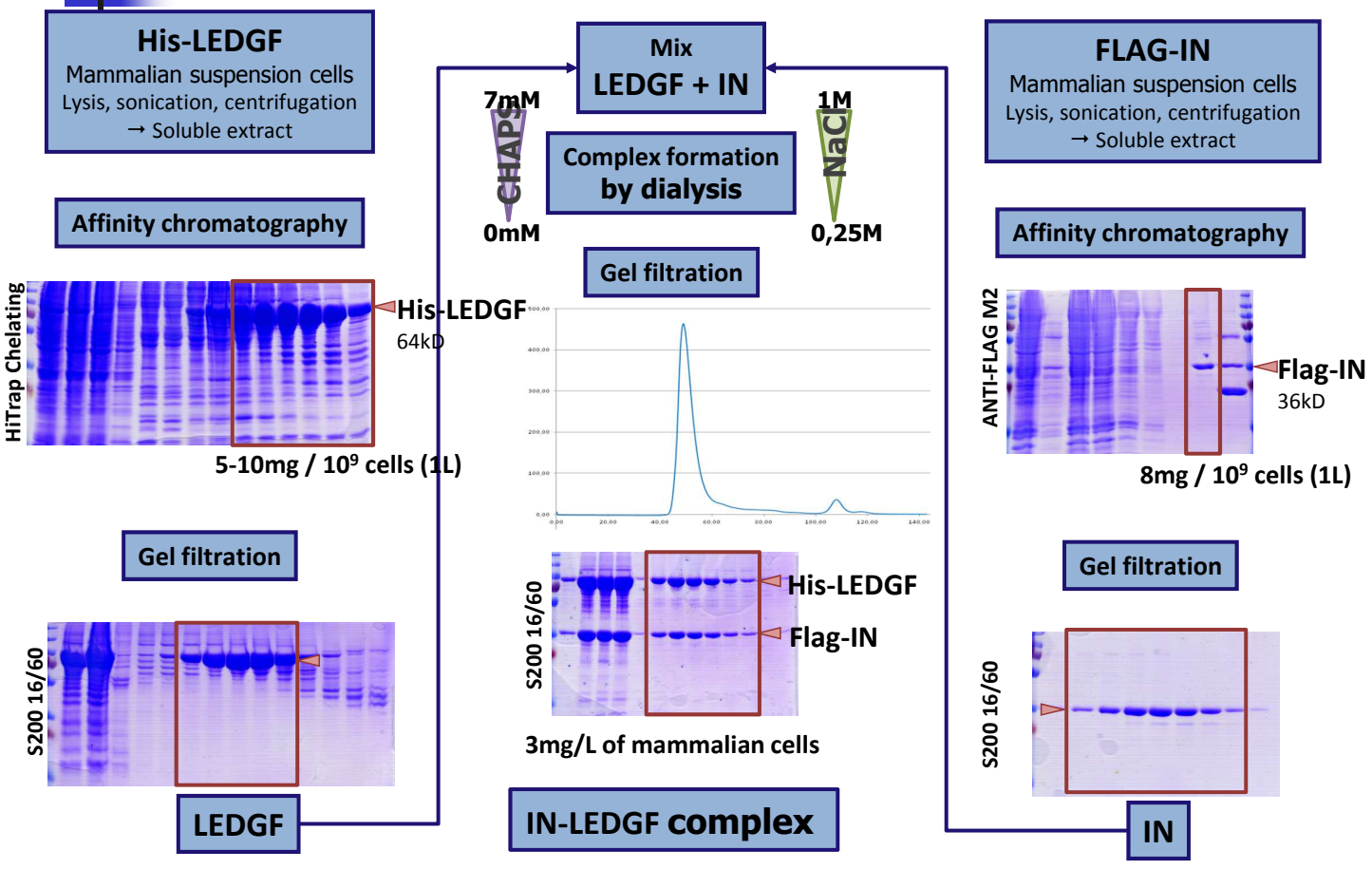
- **Vaccinia virus**: poxvirus family
  - dsDNA virus (≈ 200 kb) encoding its own transcription and replication machinery
  - viral multiplication in the cell cytoplasm: no RNA splicing
  - viral infection diverts the cellular machinery in its favour
  - at least 25 kb of foreign DNA

- **MVA**: Modified Vaccinia virus Ankara
  - non replicative in human cells
  - safe for people with immunodeficiency or skin disorders
  - manipulation is authorized under BSL1 containment

- **Mammalian cells**: BHK21 (baby hamster kidney cells)
  - authentic post-translational modifications
  - proper folding
  - protein function and structure analysis

- **Encode T7 RNA polymerase, IPTG inducible**

IN/LEDGF in mammalian cells : complex formation and purification



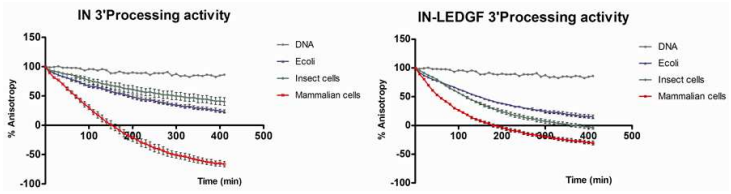
Integrase stability, solubility and dynamics

Comparison of HIV-1 IN produced in E. coli, insect and mammalian cells

Solubility

INTEGRASE	1M NaCl 7mM CHAPS	1M NaCl Ø CHAPS	0.5M NaCl Ø CHAPS
Ecoli	+	-	-
Insect cells	+	-	-
Mammalian cells	+	+	+

3' processing activity



Work in progress

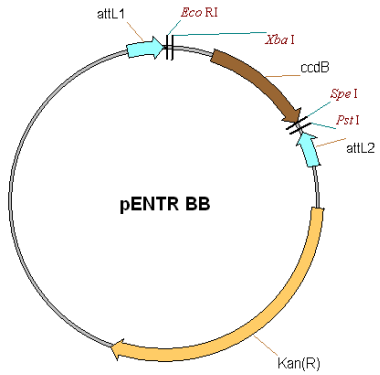
Methods development

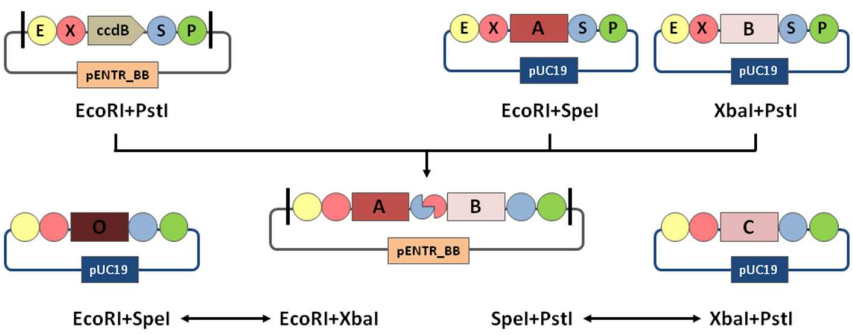
Methods developments

- **Stable complexes production**
  - Setup a synthetic biology strategy using biobricks to assemble genes and tags for protein complex expression in prokaryotic and eukaryotic cells
  - Optimize the vaccinia virus expression sytem
- **In vitro functional characterization: thermodynamic and functional characterization of IN and its complexes**
  - Functional tests using fluorescence anisotropy, fluorescence correlation spectroscopy (FCS) and fluorescence resonance energy transfer (FRET).
- **In vivo functional characterization: cellular aspects of the PIC (spatial and temporal distribution, colocation with cellular proteins)**
  - Fluorescence imaging techniques such as FRET-FLIM, FCS and high resolution fluorescence imaging with STED microscopy in collaboration with Y. Mely and the IGBMC imaging platform. Vector viruses carrying fluorescent tagged integrase (TC) will be used to infect cells transfected with plasmid carrying fluorescent cellular cofactors.
- **Non covalent complexes crystallization**
  - Setting up a pipeline of analysis procedures to decipher the best condition for the stabilization of non-covalent complexes in homogeneous conformation.
  - Development of new crystallization assays adapted to large non covalent complexes.
- **Translational Bioinformatics for AIDS**
  - Personalized medicine

Synthetic biology: biobricks for multifactorial gene assembly in expression vectors

Using synthetic biology to built complex, multi-factor expression plasmids for the optimization of protein and protein complexes expression in heterologous organisms by the combinatorial use of different DNA parts.





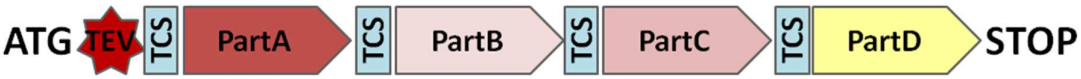
Assembly vector: New pENTR

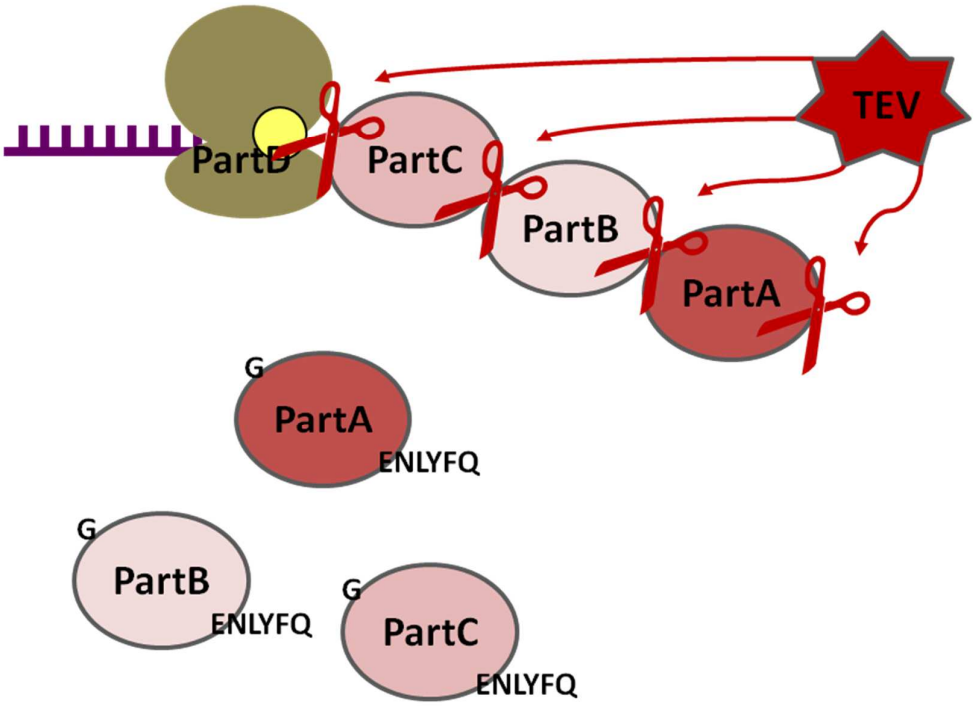
E=EcoRI, X=XbaI, S=SpeI, P=PstI, N=Not1

Ho-Shing et al., 2012, Methods in Molecular Biology, 852, 61-76

http://partsregistry.org
http://biobricks.org/

Biobrick for protein complexes expression in E. Coli, Insect and mammalian cells

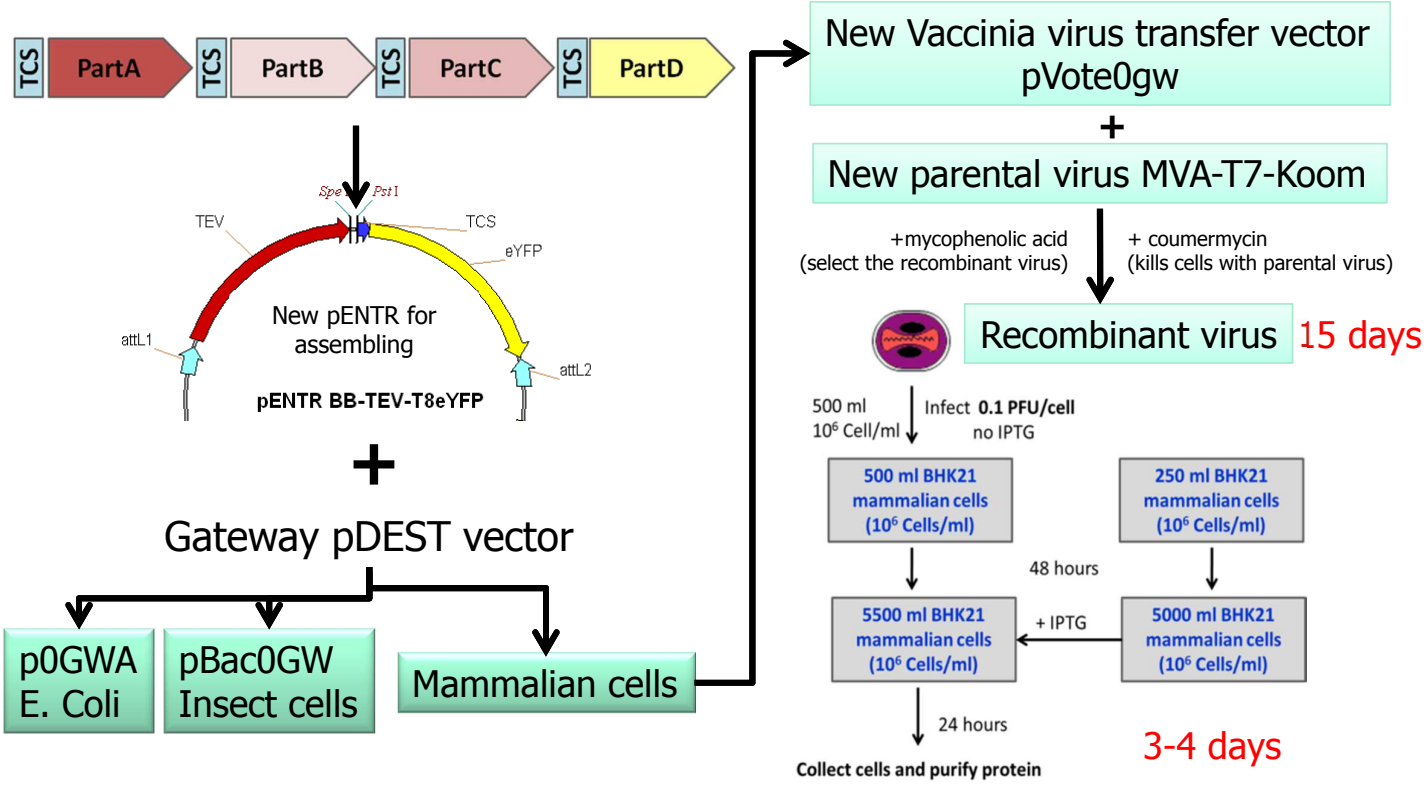




Bieniossek et al., Trends in Biochemical Sciences 2012;37(2):49-57

Mammalian cell protein complexes production

Optimization of the Modified Ankara Vaccinia virus as an expression vector for protein production in BHK21 mammalian cells

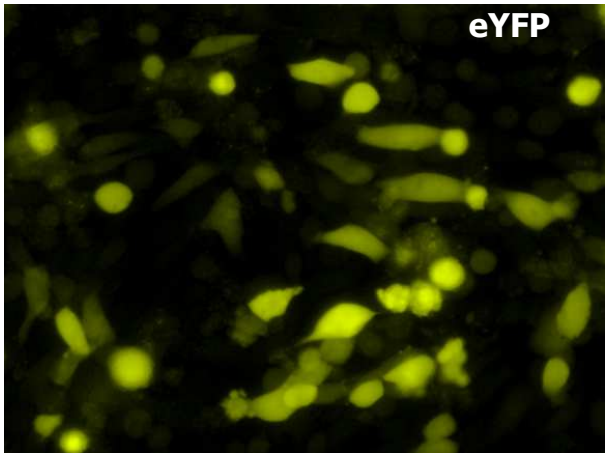


Biobrick collection for protein expression in E. Coli, Insect and mammalian cells

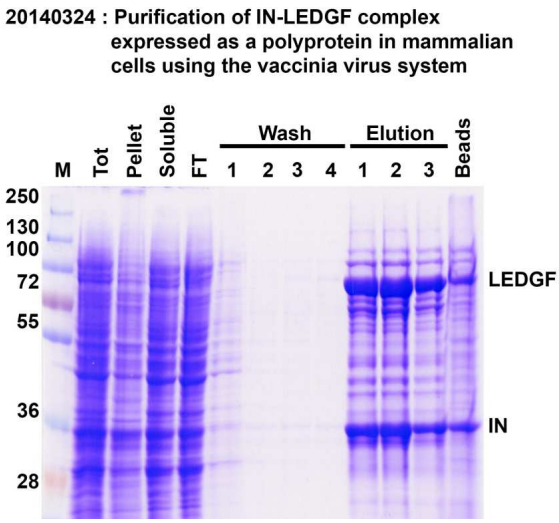
Purification tags	H6P	6xHis - P3C cleavage site
	H10P	10xHis - P3C cleavage site
	H10FP	10xHis - Flag- P3C cleavage site
	FH10P	Flag - 10xHis - P3C cleavage site
	RP	Strep - P3C cleavage site
	RRP	Strep - Strep - P3C cleavage site
	RRFP	Strep - Strep - Flag - P3C cleavage site
	FRRP	Flag - Strep - Strep - P3C cleavage site
TEV cleavage sites	TT1	Twin TEV cleavage site (TCS) 1 : TCS A – TCS B
	TT2	Twin TEV cleavage site (TCS) 2 : TCS C – TCS D
	TT3	Twin TEV cleavage site (TCS) 3 : TCS E – TCS F
	TT4	Twin TEV cleavage site (TCS) 4 : TCS G – TCS H
Linker STOP	STOP	STOP codon
Proteins	TEV	Tobacco Etch Virus protease
	LEDGF	Human Lens epithelial derived growth factor
	IN	HIV-1 Integrase
	IN*	Degenerated HIV-1 Integrase
	TNPO3	Human Transportin
	TT8-eYFP	Twin TEV cleavage site 8 – enhanced Yellow Fluorescent Protein
	……	

System validation: IN/LEDGF complex production in mammalian cells

Poly-protein expression validation eYFP expression

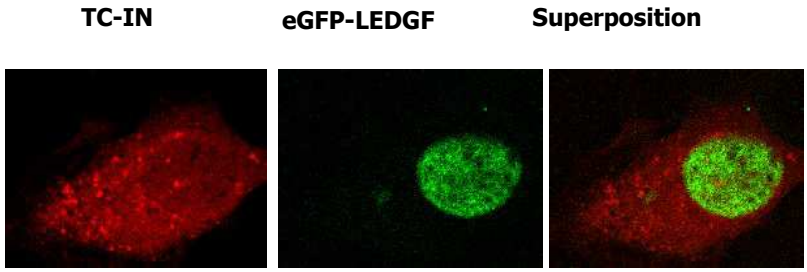


IN-LEDGF complex purification



In vivo Functional Characterization (FRET-FLIM)

- 1. Co-transfection of TC-Integrase + LEDGF-EGFP plasmids (confocal microscope, fixed cells)



- 2. Infection with HIV-1 VSVG pseudotyped virus and transfection with LEDGF-EGFP plasmid. Knock down of endogenous LEDGF by shRNA (confocal and STED microscope, fixed and live cells)

Non covalent complexes Crystallization

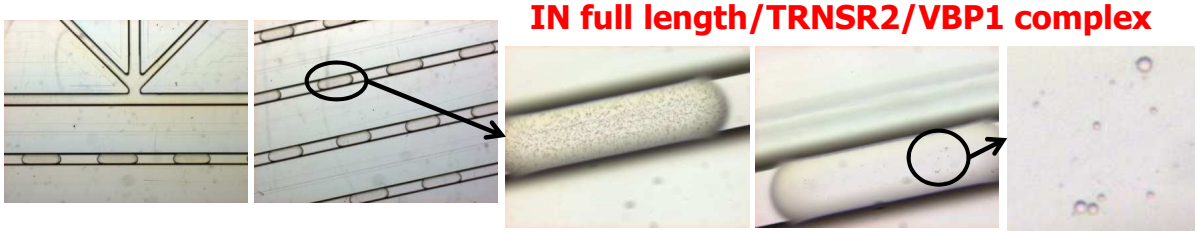
Nanodrop crystallization techniques (vapor diffusion)
(Drop setup with Mosquito robot and visualization with Rock Maker)

IN full length / LEDGF IBD complex

Plate screening on beam line
Crystal harvesting

Microfluidics crystallization techniques (Plug-Maker, Protein Biosolution)

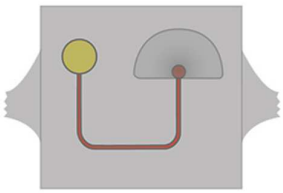
IN full length/TRNSR2/VBP1 complex



Crystal harvesting

Microfluidics crystallization techniques (Crystal Former, Microlytic)

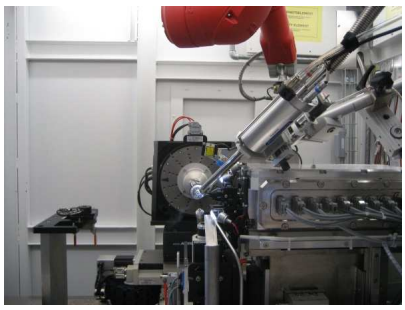
96 well microfluidic device

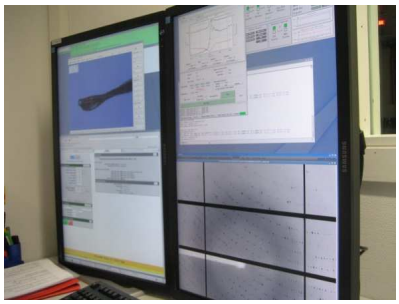


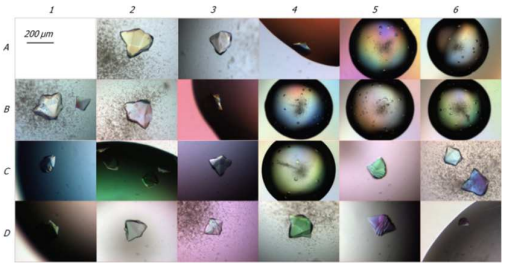
Crystallization in progress

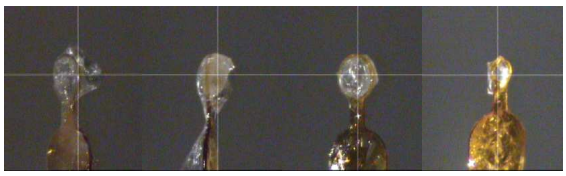
Plate screening on beam line

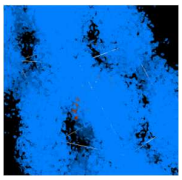
Crystal harvesting

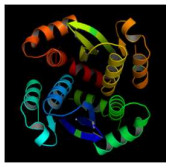






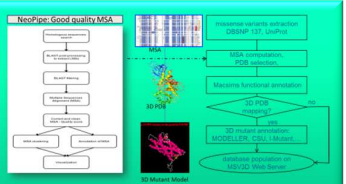






Development of Translational Bioinformatics for AIDS

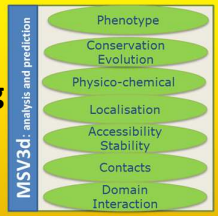
Structural data
Atomic structures
Drug resistance mutants
Multiple sequences alignment
Clinical data: HTP sequencing



Physico-chemical data
Proteins stability
Proteins solubility
…

Phenotypic data
OMIM :list of known human genetic disease
GO: gene information
CLINICAL data
…

Development of a single comprehensive database from heterogeneous sources describing HIV variants (based on MSV3D describing human variants (800000))



Functional data
In cellulo data
Enzymatic activities
Binding constants
Interaction networks
…

Molecular mechanisms of viral replication

HTP structure modelisation
↓
HTP Small molecules docking
↓
Personalized medicine

Full access by the community
http://decrypthon.igbmc.fr/sm2ph/cgi-bin/home
http://decrypthon.igbmc.fr/msv3d/cgi-bin/home
Dedicated restricted databases

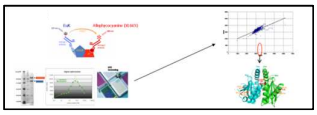
Luu, T.D.,Rusu, A.M., Walter, V., Ripp, R.,Moulinier, L.,Muller, J., Toursel, T.,Thompson,J., Poch, O.,and Nguyen,H. (2012). MSV3d: database of human MisSense variants mapped to 3D protein structure. Database (Oxford) *2012*

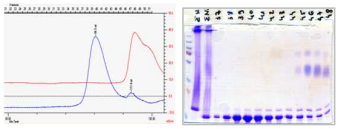
Nguyen H, Laurent M, Thompson JD, Poch O (2014). Heterogeneous Biological Data Integration with High Level Query Language. IBM Journal of Research and Development, vol. 58 no. 2/3 , 15 April, 2014. doi: 10.1147/JRD.2014.2309032.

DRUG-DESIGN

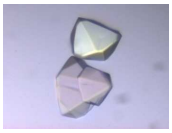
Protein – Protein interaction and allosteric inhibitors

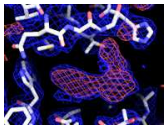
HTP IN – LEDGF interaction inhibitors screening (HTRF)





IN CCD Production and crystallization

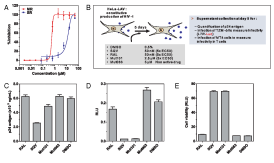




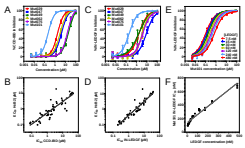
HTP inhibitors crystal soaking, data collection, structure

70 structures solved

Structure based drug design

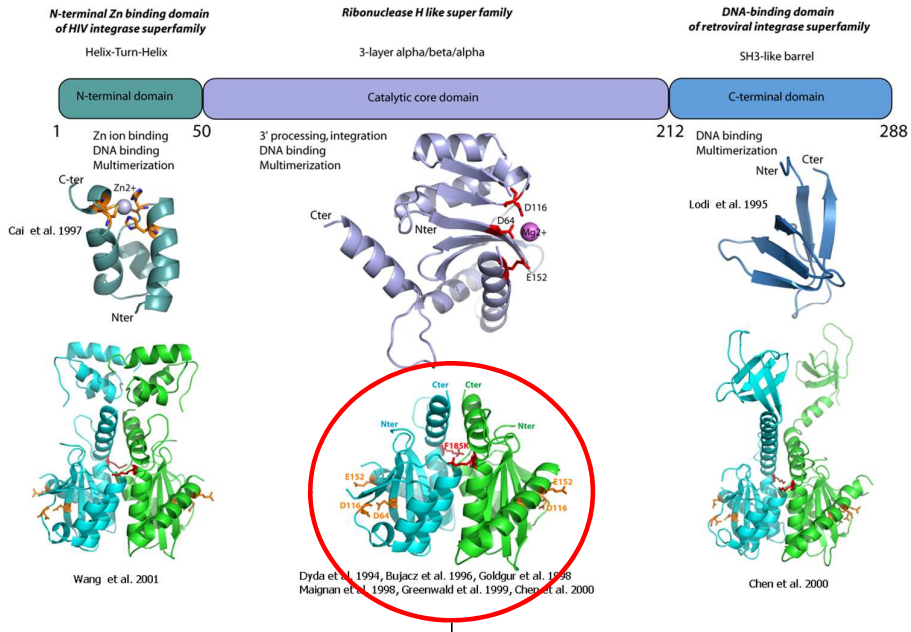


in-vitro functional assay



In cellulo functional assay

HIV Integrase



CONSTRUCT	MUTANT	SEQ.	VECTOR	MW	MW fusion	MW protein	PI	PI fusion
His-tb-INTEGRASE	F185K	50-212	pEt15b	19,9	1,9	18,0	8,05	10,54

Crystallization of IN CCD for Drug Design

Production of IN CCD [50-212]
E.Coli – 4h at 37°C

↓

Affinity purification
Nickel column

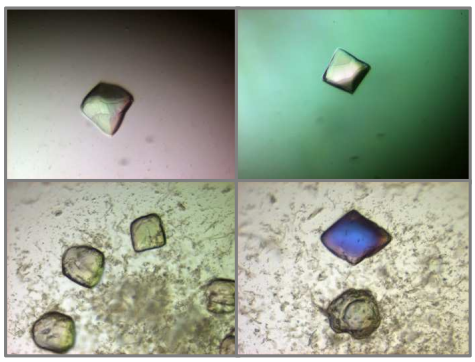
↓

Size exclusion chromatography
Superdex 200

↓

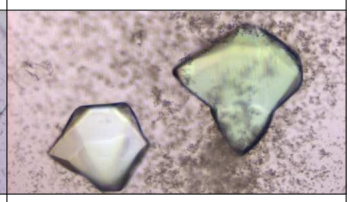
Crystallization
1.26 M AmSO4
100 mM NaCacodylate pH 6.5
Hanging drops
@ 22-23 °C

22-23°C
Diffraction
~2Å

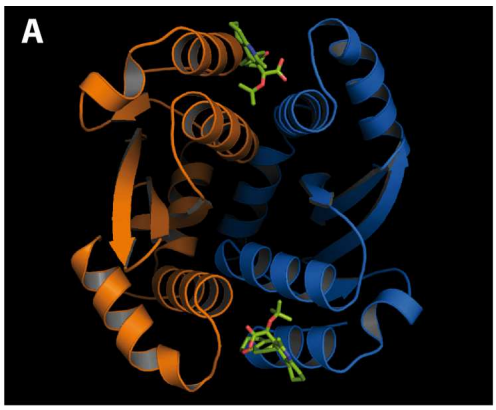


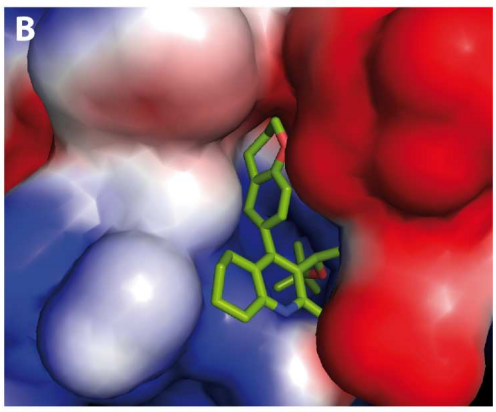
24-25°C
No diffraction !

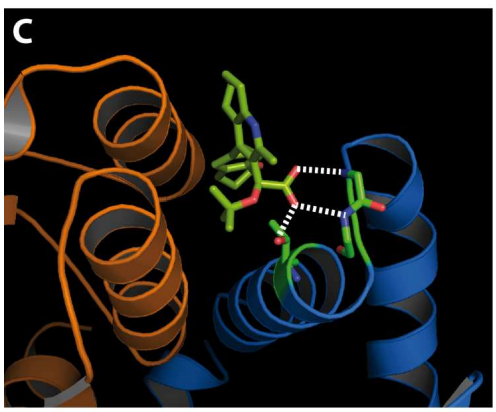
HIV Integrase 50 - 212 (F185K), Crystallization

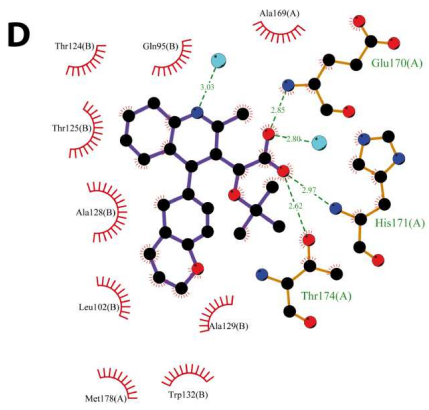
Crystal name	P1_B2_L11	P1_D4_L12	P1_C6_L13
Crystal source	Plate1_B2	Plate1_D4	Plate1_C6
Crystal image			
Protein concentration	3.1 mg/ml	3.1 mg/ml	3.1 mg/ml
Reservoir composition	1.26 M AS	1.26 M AS	1.50 M AS
Initial drop composition	2µl prot + 2µl res	2µl prot + 2µl res	2µl prot + 2µl res
Ligand soaking	LIG11	LIG12	LIG13

HIV Integrase CCD – ligand structure

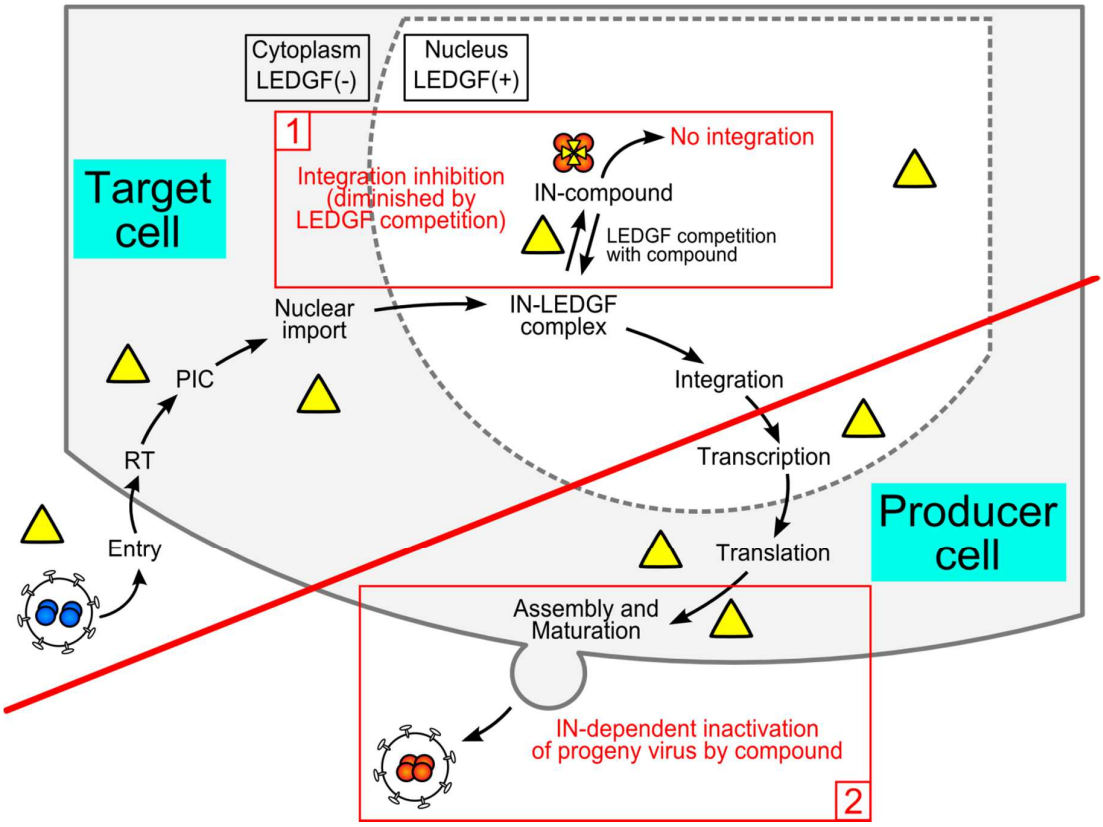




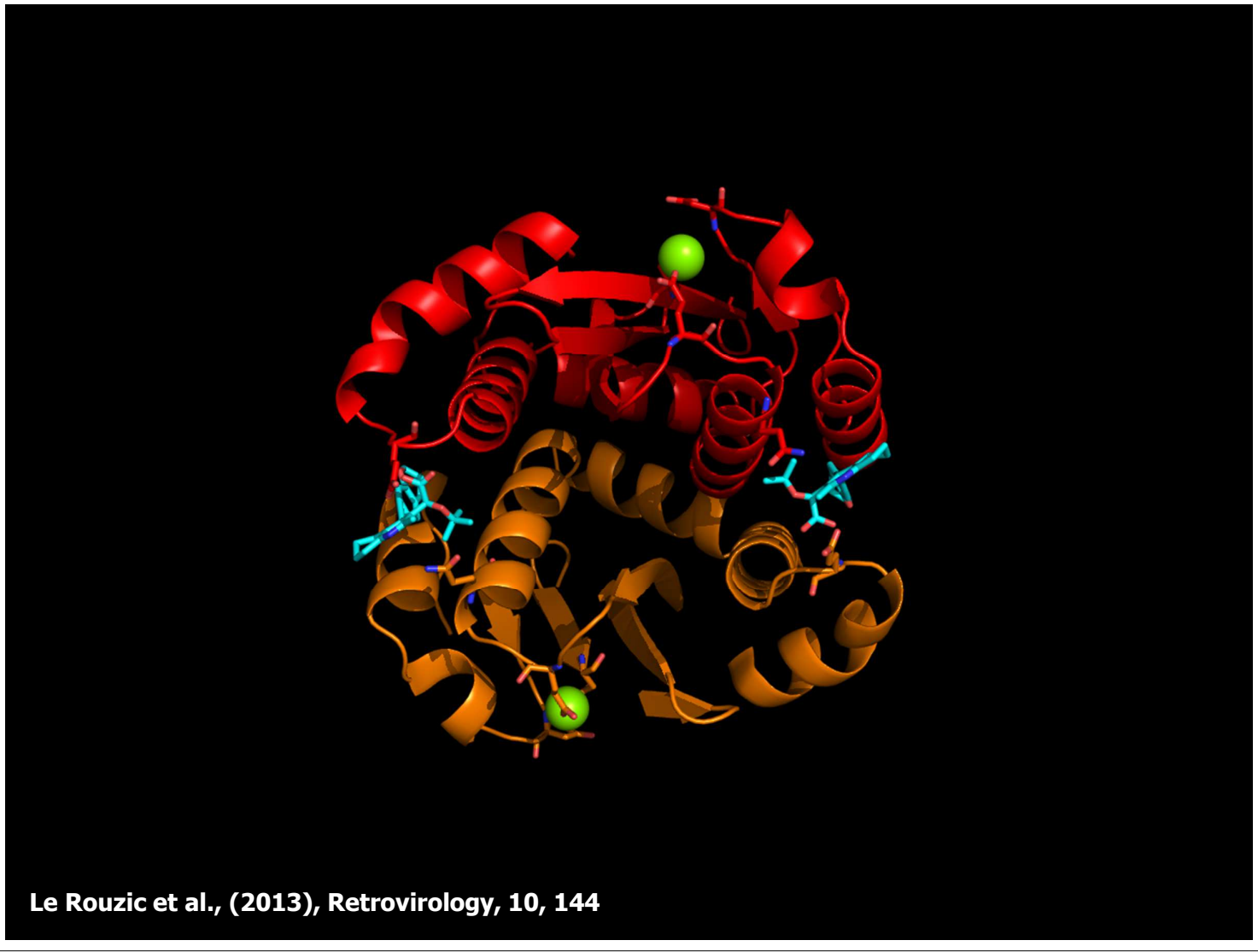




IN – LEDGF interaction and IN allosteric inhibitors

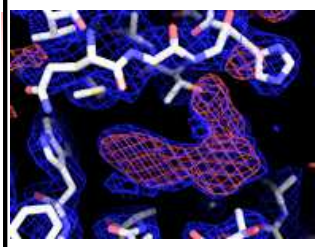


Le Rouzic et al., (2013), Retrovirology, 10, 144



Le Rouzic et al., (2013), Retrovirology, 10, 144

INLAIs inhibitors



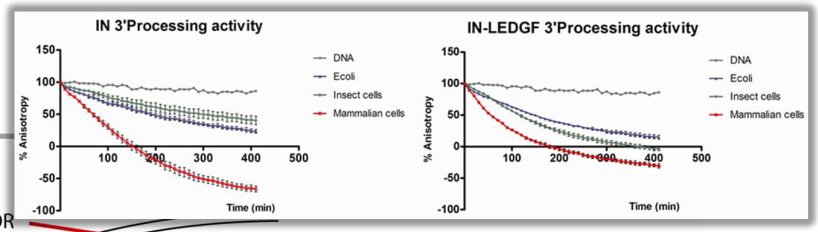
70 structures solved

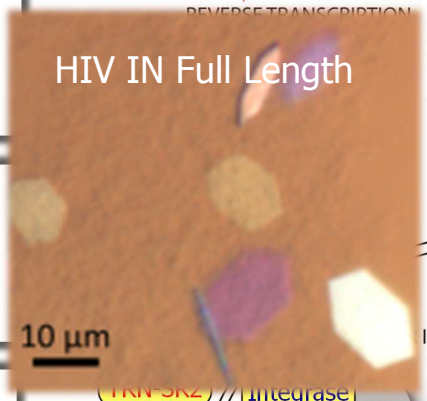
Allosteric inhibitors. The allosteric effects are mediated by structural changes in the active site, dimeric interface, accessible surface.

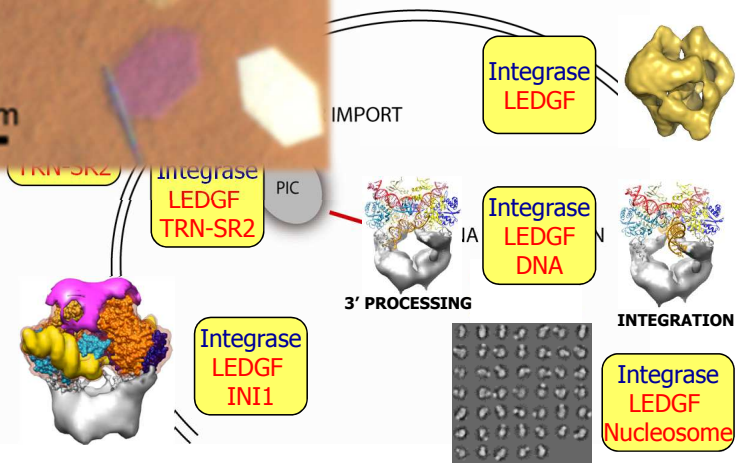
Mammalian cells production: increase integrase stability, solubility and 3'processing activity. Presence of acetylation and phosphorylation

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







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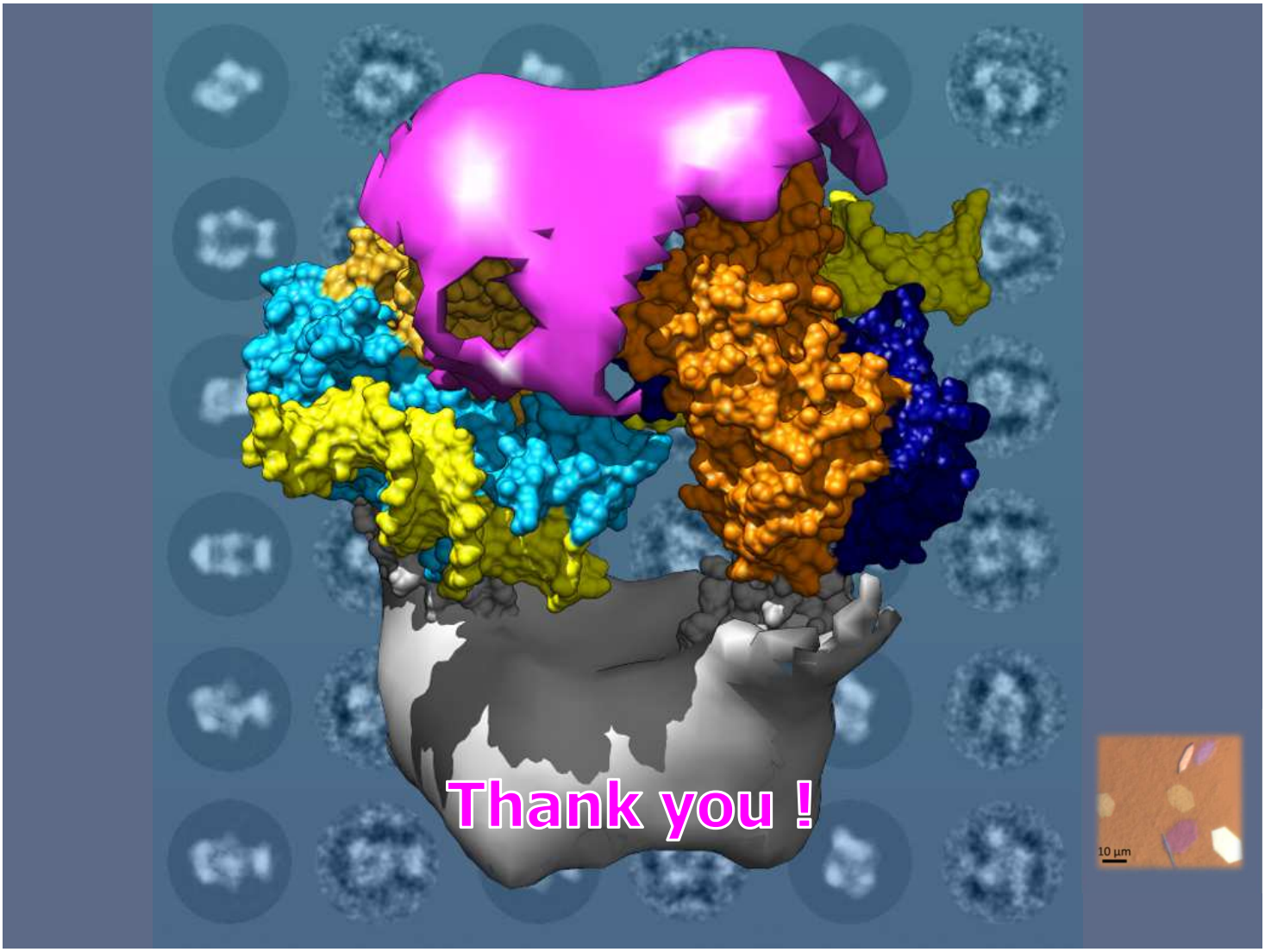
Sarah Lennon

Members of the IGBMC Mass Spectrometry Facility
Members of Structural Biology and Genomics platform
CEGBS, Illkirch
Members of the bioinformatic group and platform
Members of the imaging platform
Members of the IGBMC's common services
Cell, baculovirus and cloning facilities


Thank you !
10 µm