IGBMC
Institut de génétique et de biologie moléculaire et cellulaire

FROM UNSTABLE PROTEIN TO STABLE COMPLEXES
THE HIV-1 PRE-INTEGRATION COMPLEX, STEROID NUCLEAR RECEPTORS
STRUCTURE, FUNCTION AND DYNAMICS

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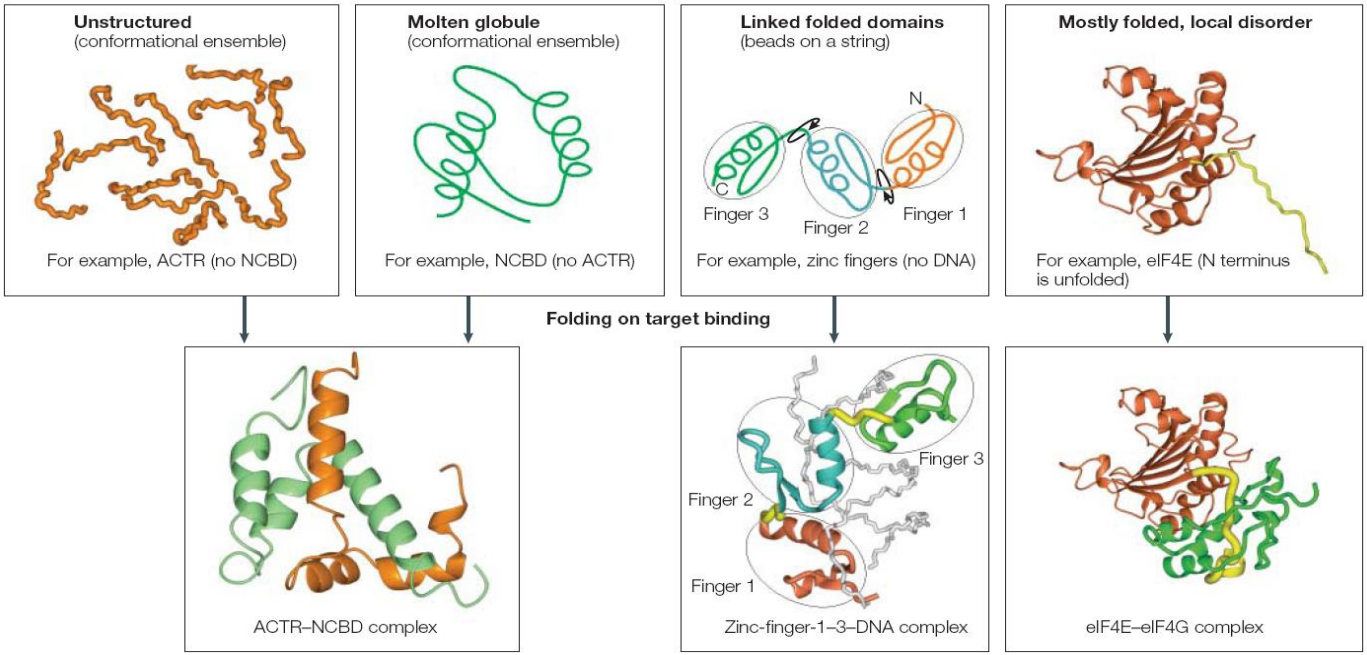
Intrinsically Disordered Proteins (IDP)

Definitions: (Keith Dunker - Indiana University)

Many proteins contain regions that lack specific 3-D structure; indeed some proteins lack specific 3-D structure in their entireties under physiological conditions and yet carry out biological function. Such proteins and regions have been called natively unfolded, intrinsically unstructured, naturally disordered, …



H. Jane Dyson and Peter E.Wright, Nature Reviews In Molecular Cell Biology, 2005, 6, 197-208

Intrinsic disorder and protein interaction networks

Dunker et al, Flexible nets: The roles of intrinsic disorder in protein interaction networks, FEBS J. 2005, 272, 5129-5148)

- plasticity of the disorder regions allows the binding of multiple partners
- Few proteins bind to many partners (Hub proteins) but most proteins bind to few partners
- Many disordered regions are modified post-transcriptionaly (Phosphorylation, Acetylation, ubiquitination, proteolytic cleavage)

Intrinsically disordered proteins: function, folding, and flexibility

IDPs participate in important regulatory functions in the cell, including transcription, translation, the cell cycle, and numerous signal transduction events. Disordered proteins often undergo coupled folding and binding transitions upon interaction with their cellular targets.

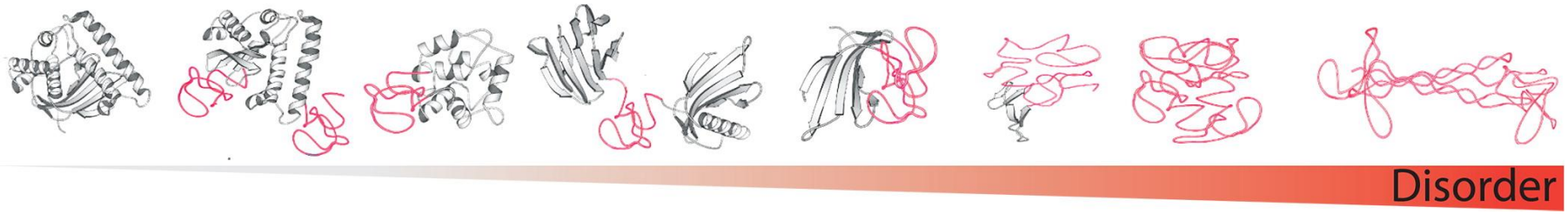
The lack of stable globular structure confers numerous functional advantages, but not without cost; many disordered proteins are associated with amyloid disease and with chromosomal translocations in cancer.

Intrinsically disordered proteins occupy a continuum of "conformational space", ranging from highly unstructured, through molten globule, to local disorder within an otherwise folded domain.

Intrinsically disordered proteins: function, folding, and flexibility

While many proteins need to adopt a well-defined structure to carry out their function, a large fraction of the proteome of any organism consists of polypeptide segments that are not likely to form a defined three-dimensional structure, but are nevertheless functional. These protein segments are referred to as **intrinsically disordered regions (IDRs)**.

Proteins sequences in a genome can be viewed as modular because they are made up of combinations of structured and disordered regions. **Proteins without IDRs are called structured proteins, and proteins with entirely disordered sequences that do not adopt any tertiary structure are referred to as intrinsically disordered proteins (IDPs).** The majority of eukaryotic proteins are made up of both structured and disordered regions, and both are important for the repertoire of functions that a protein can have in a variety of cellular contexts.

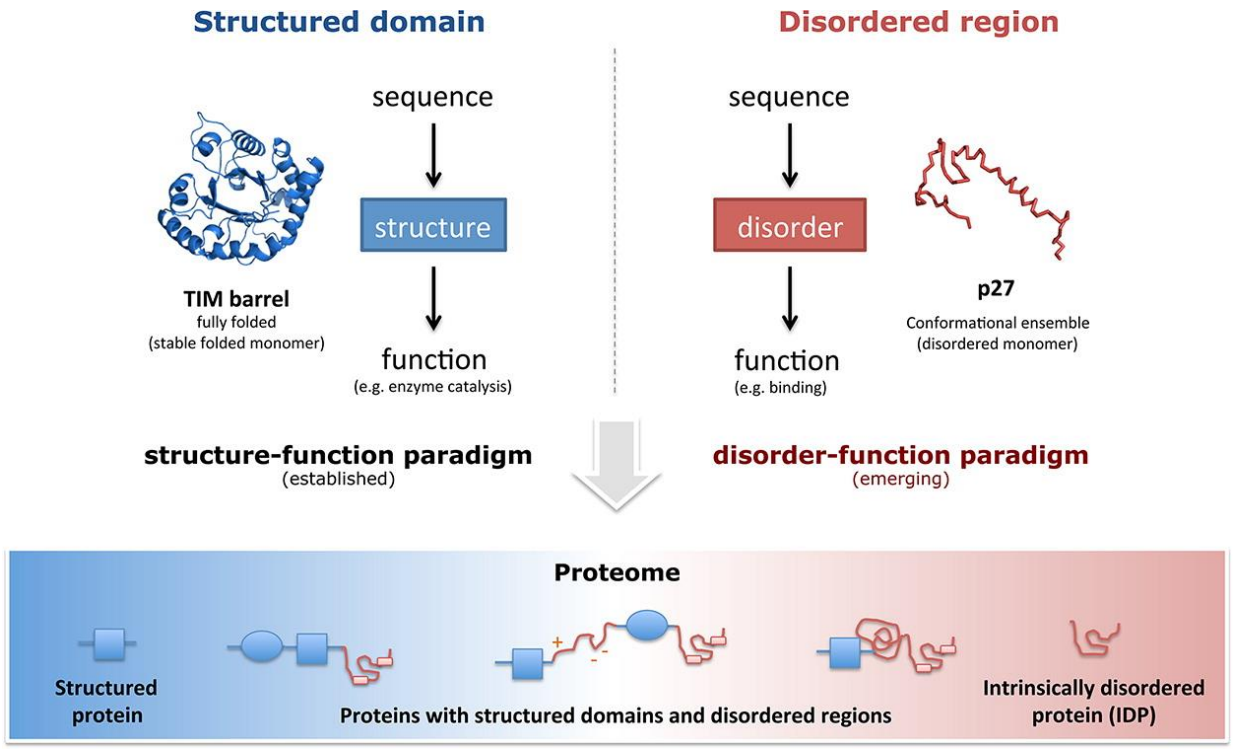


IDRs and IDPs are prevalent in eukaryotic genomes. For instance, 44% of human protein-coding genes contain disordered segments of >30 amino acids in length.

Johnny Habchi; Peter Tompa; Sonia Longhi; Vladimir N. Uversky; *Chem. Rev.* **2014,** 114, 6561-6588.

Robin van der Lee; Marija Buljan; Benjamin Lang; Robert J. Weatheritt; Gary W. Daughdrill; A. Keith Dunker; Monika Fuxreiter; Julian Gough; Joerg Gsponer; David T. Jones; Philip M. Kim; Richard W. Kriwacki; Christopher J. Oldfield; Rohit V. Pappu; Peter Tompa; Vladimir N. Uversky; Peter E. Wright; M. Madan Babu; *Chem. Rev.* **2014,** 114, 6589-6631. DOI: 10.1021/cr400525m.

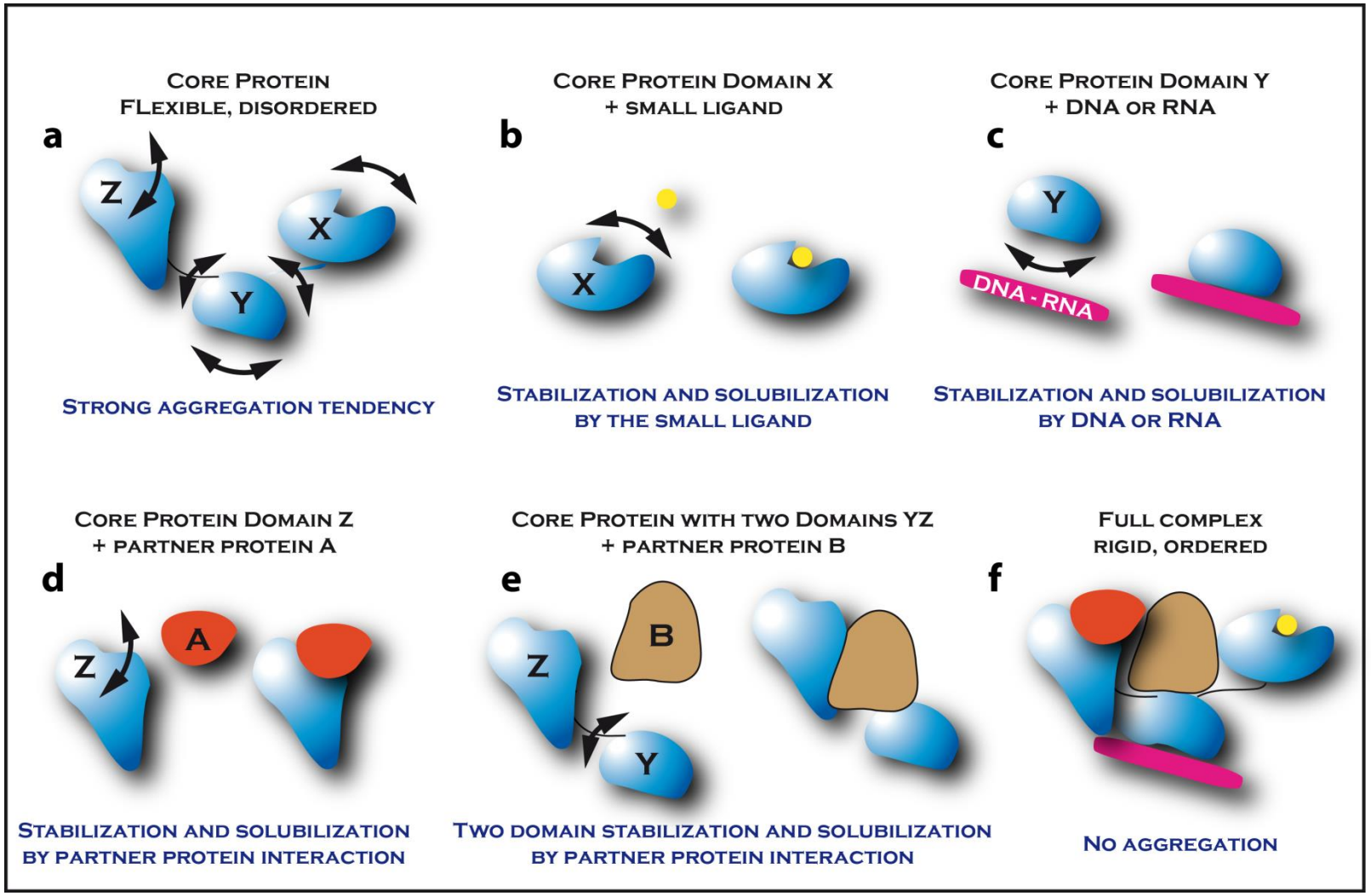
Intrinsically disordered proteins: function, folding, and flexibility



Structured domains and intrinsically disordered regions (IDRs) are two fundamental classes of functional building blocks of proteins. The synergy between disordered regions and structured domains increases the functional versatility of proteins.

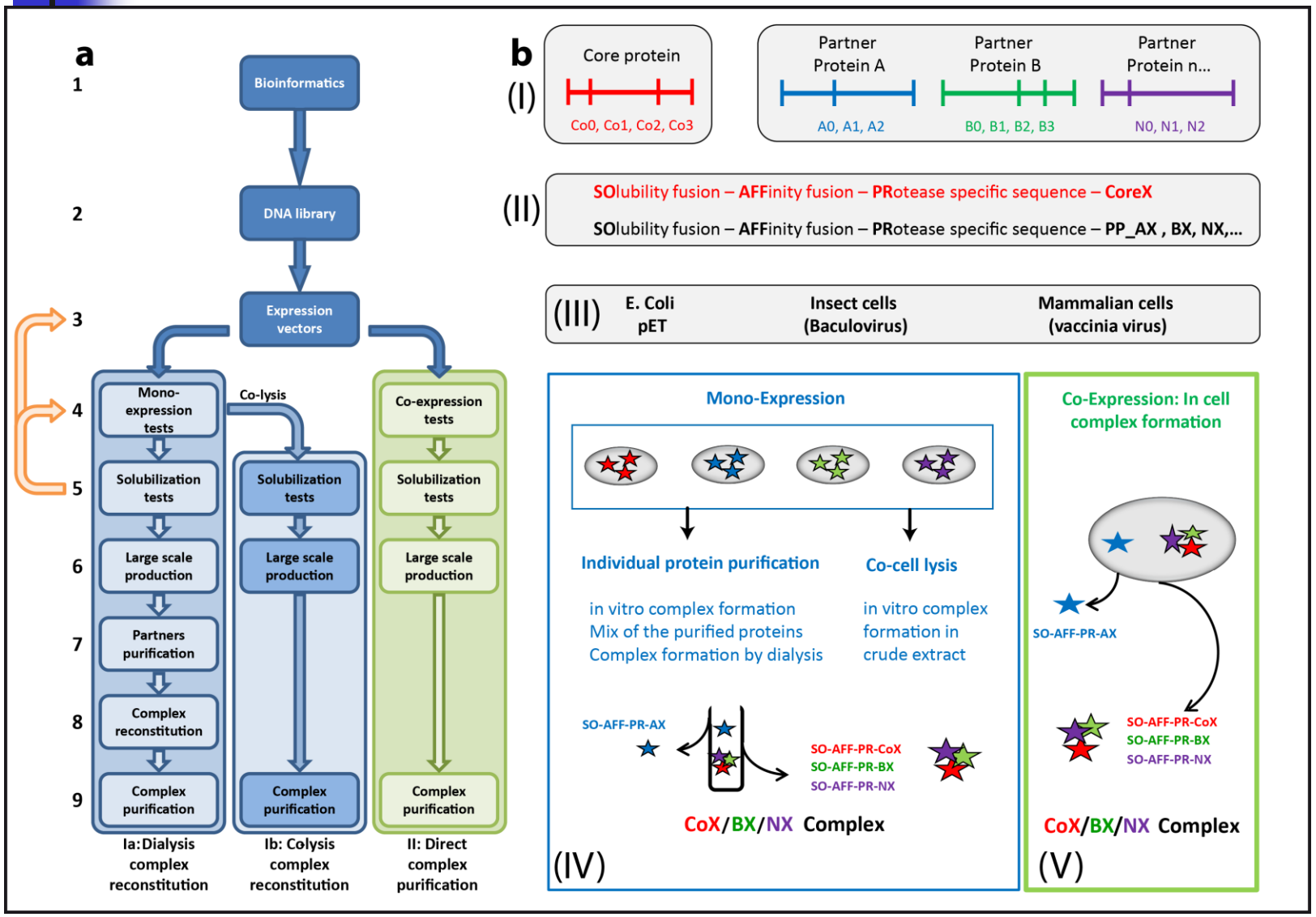
Robin van der Lee; Marija Buljan; Benjamin Lang; Robert J. Weatheritt; Gary W. Daughdrill; A. Keith Dunker; Monika Fuxreiter; Julian Gough; Joerg Gsponer; David T. Jones; Philip M. Kim; Richard W. Kriwacki; Christopher J. Oldfield; Rohit V. Pappu; Peter Tompa; Vladimir N. Uversky; Peter E. Wright; M. Madan Babu; *Chem. Rev.* **2014,** 114, 6589-6631. DOI: 10.1021/cr400525m.

From unstable protein to stable complexes



Levy N, Eiler S, Pradeau-Aubreton K, Maillot B, Stricher F, Ruff M (2016). Nature Communications 7: 10932

From unstable protein to stable complexes



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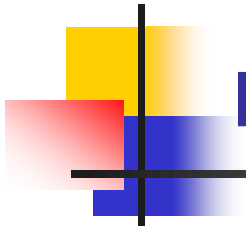
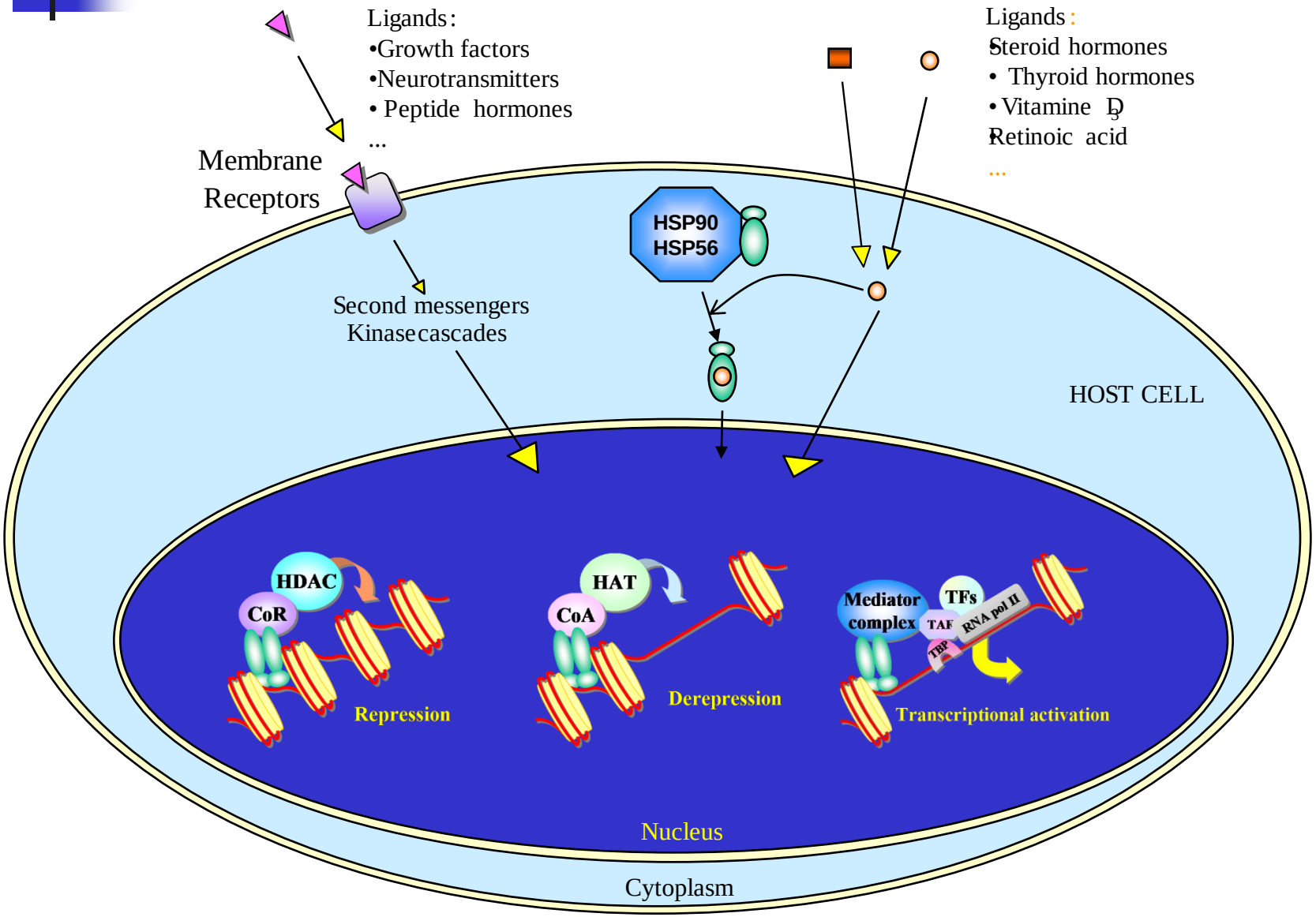


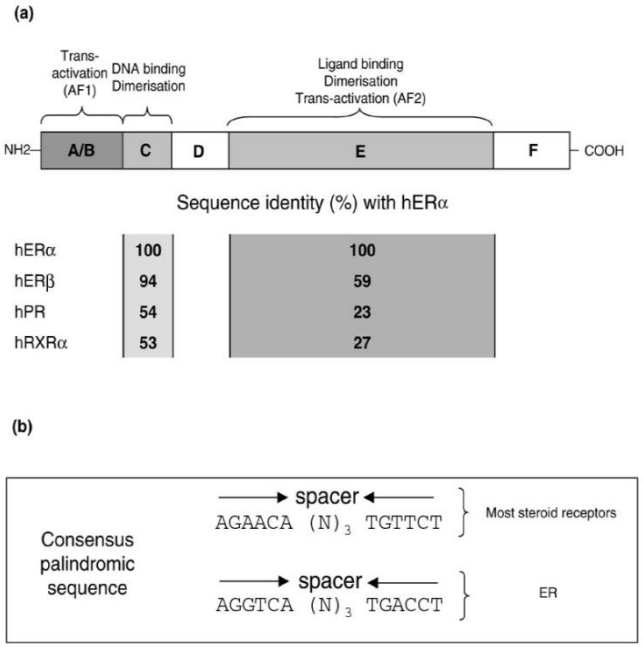
Example 1

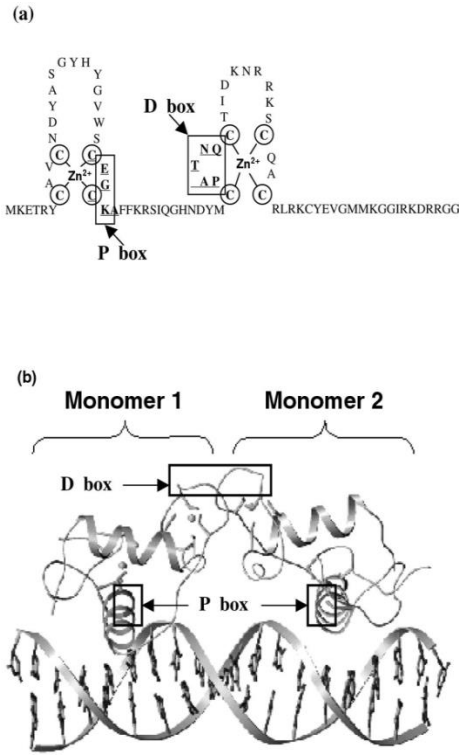
Steroid Hormones Nuclear Receptors

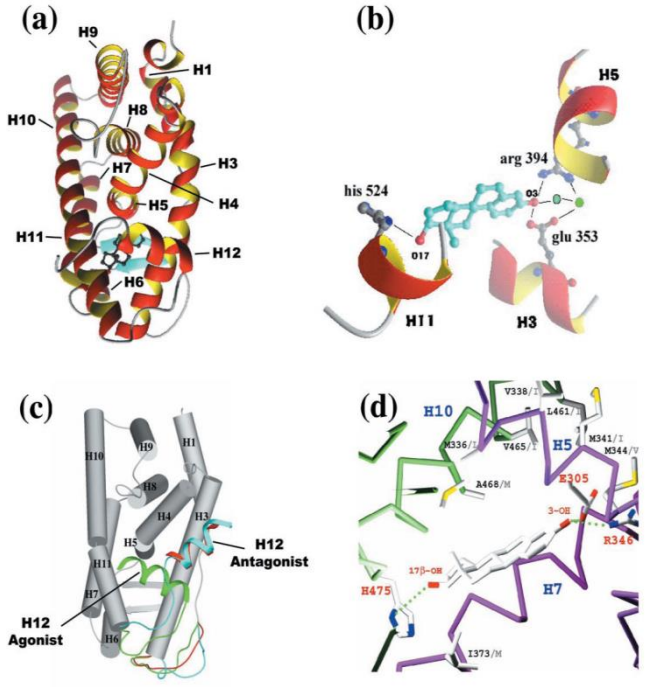
Steroid hormone nuclear receptors



Steroid nuclear receptors organized into domains with IDRs

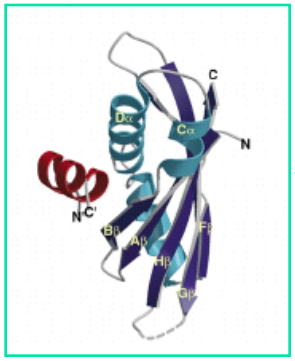




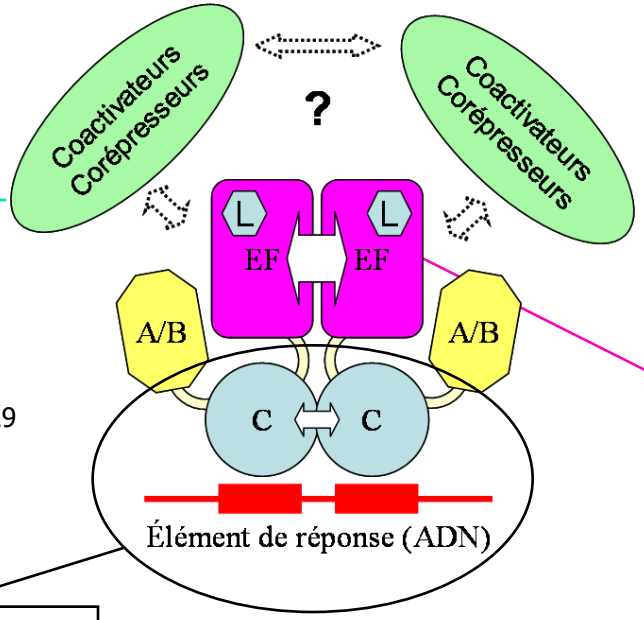


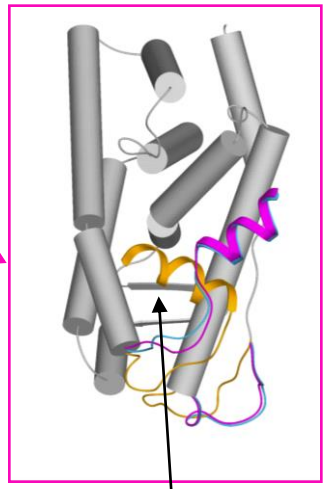
Marc Ruff, Monique Gangloff, Jean Marie Wurtz and Dino Moras. Estrogen receptor transcription and transactivation. Structure–function relationship in DNA- and ligand-binding domains of estrogen receptors. *Breast Cancer Res* 2000, **2**:353–359

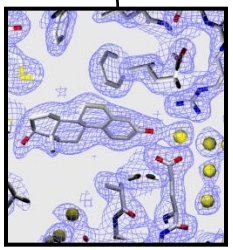
Steroid hormone nuclear receptors

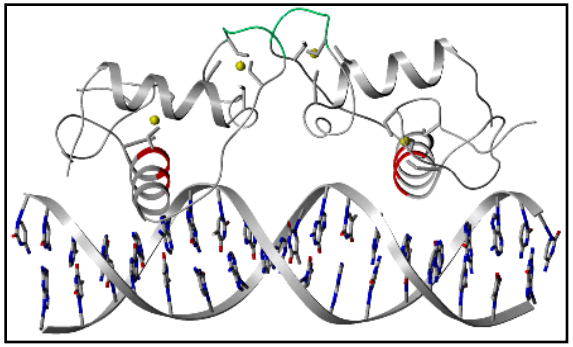


Razeto et al, (2004), J. Mol. Biol. 336, 319-329







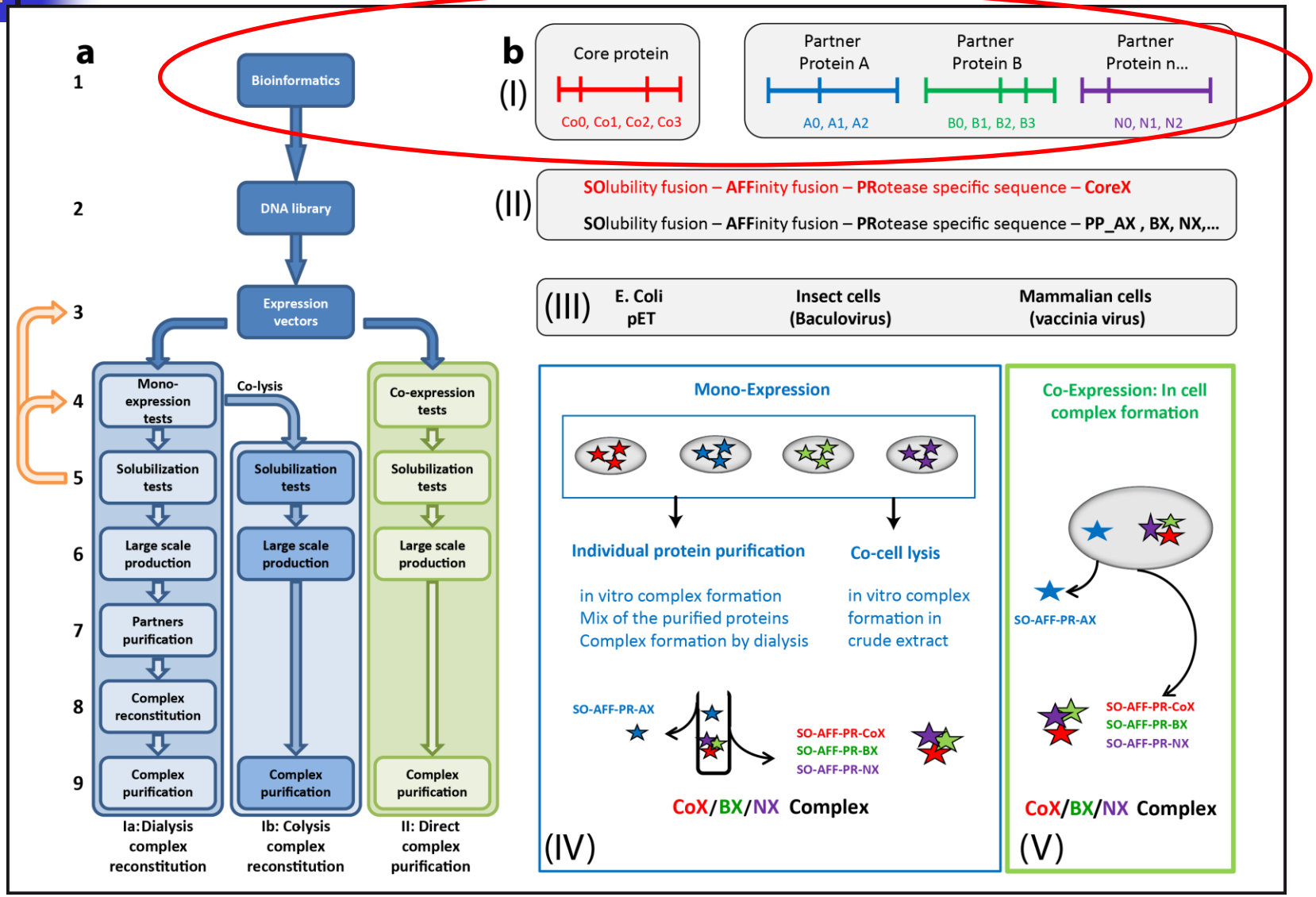


Luisi et al, (1991). Nature *352*, 497-505.

Gangloff et al, (2001), JBC, 276, 15059-15065.

→ Interaction of SNRs with TIF2 coactivator

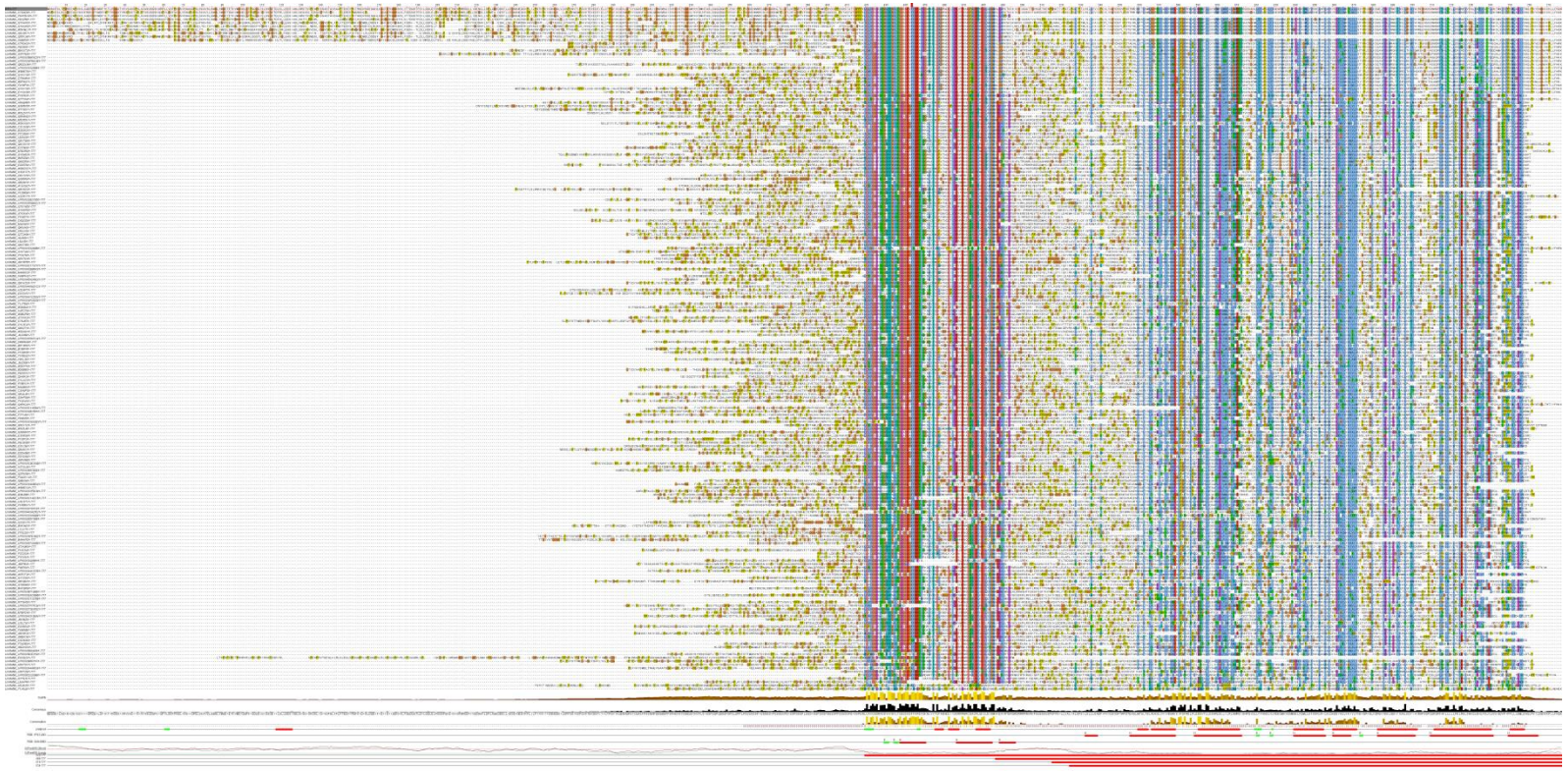
From unstable protein to stable complexes



Levy N, Eiler S, Pradeau-Aubreton K, Maillot B, Stricher F, Ruff M (2016). Nature Communications 7: 10932

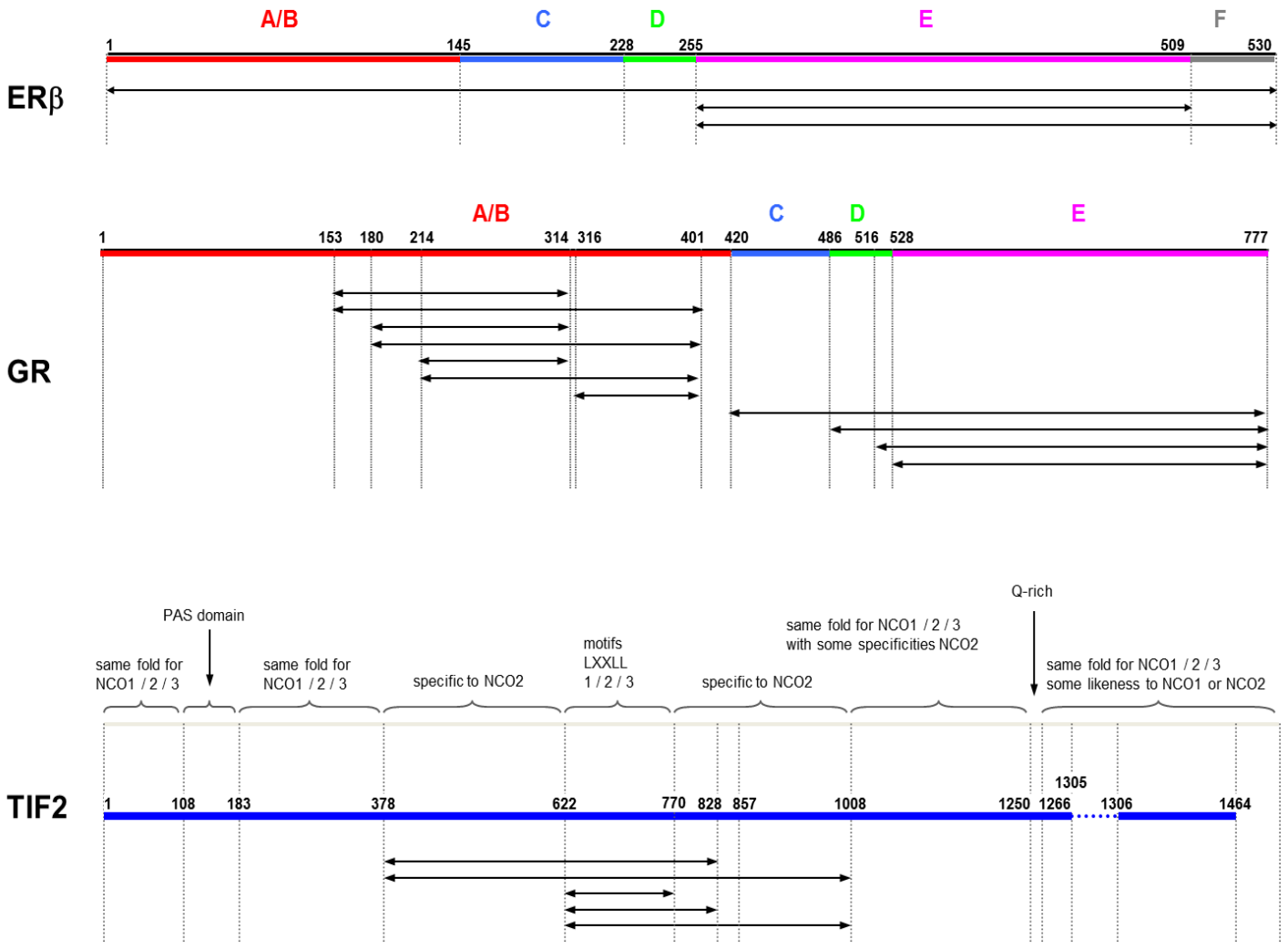
Bioinformatics analysis of the sequences: design of constructs

GR multi – alignment (jalview)

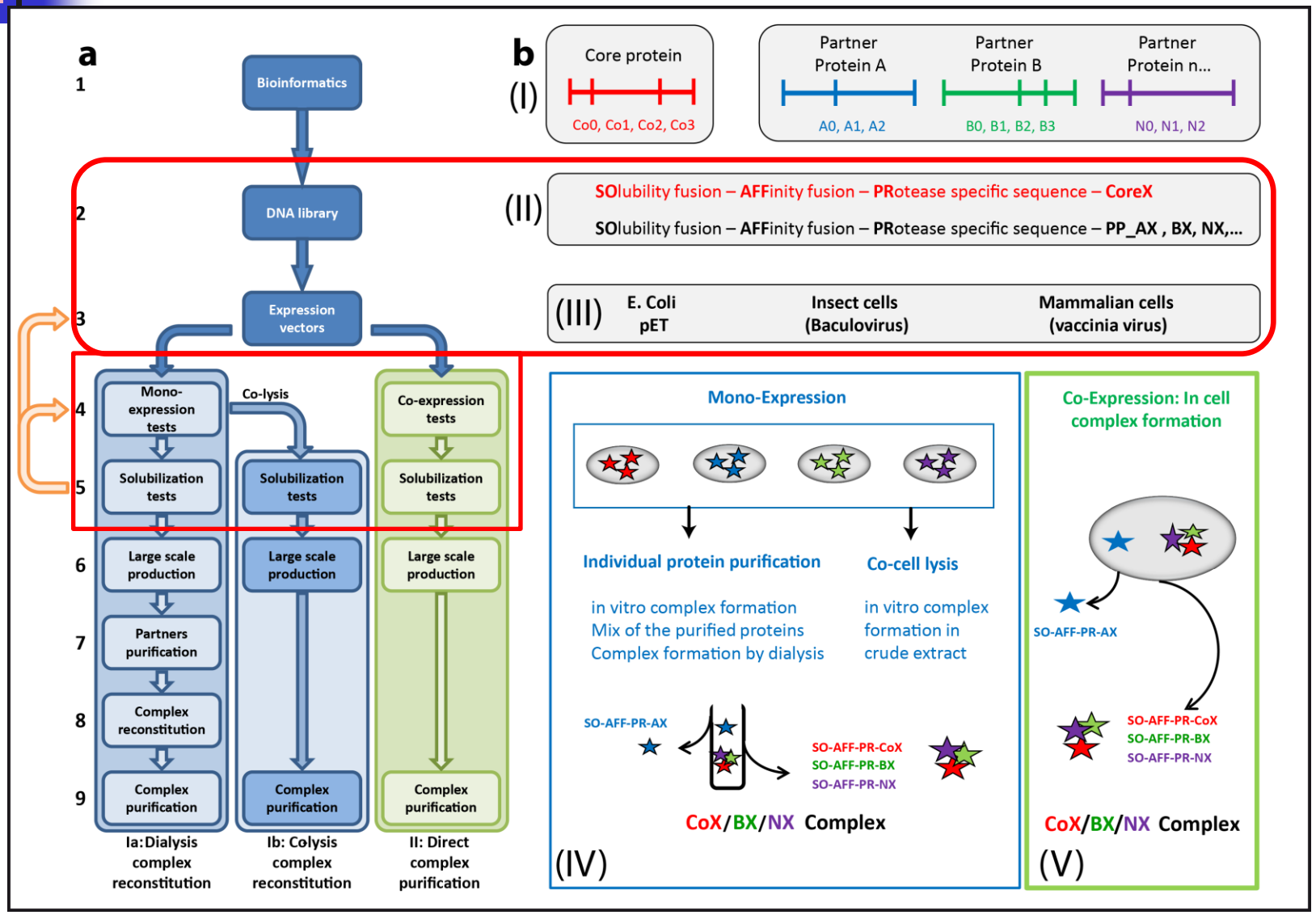


TIF2 multi - alignment

Bioinformatics analysis of the sequences of ERß GR and TIF2 : design of constructs



From unstable protein to stable complexes



Levy N, Eiler S, Pradeau-Aubreton K, Maillot B, Stricher F, Ruff M (2016). Nature Communications 7: 10932

ERβ Expression and solubilisation tests

E. Coli expression

ERβ	HIS		GST		HIS-TRX		HIS-MBP	
	LBS/18°C		LBS/18°C		LBS/18°C		LBS/18°C	
	T	S	T	S	T	S	T	S
FL 1 – 530	++	-	++	-	++	-	++	-
EF 255 – 530	nd	nd	nd	nd	+++	++	nd	nd

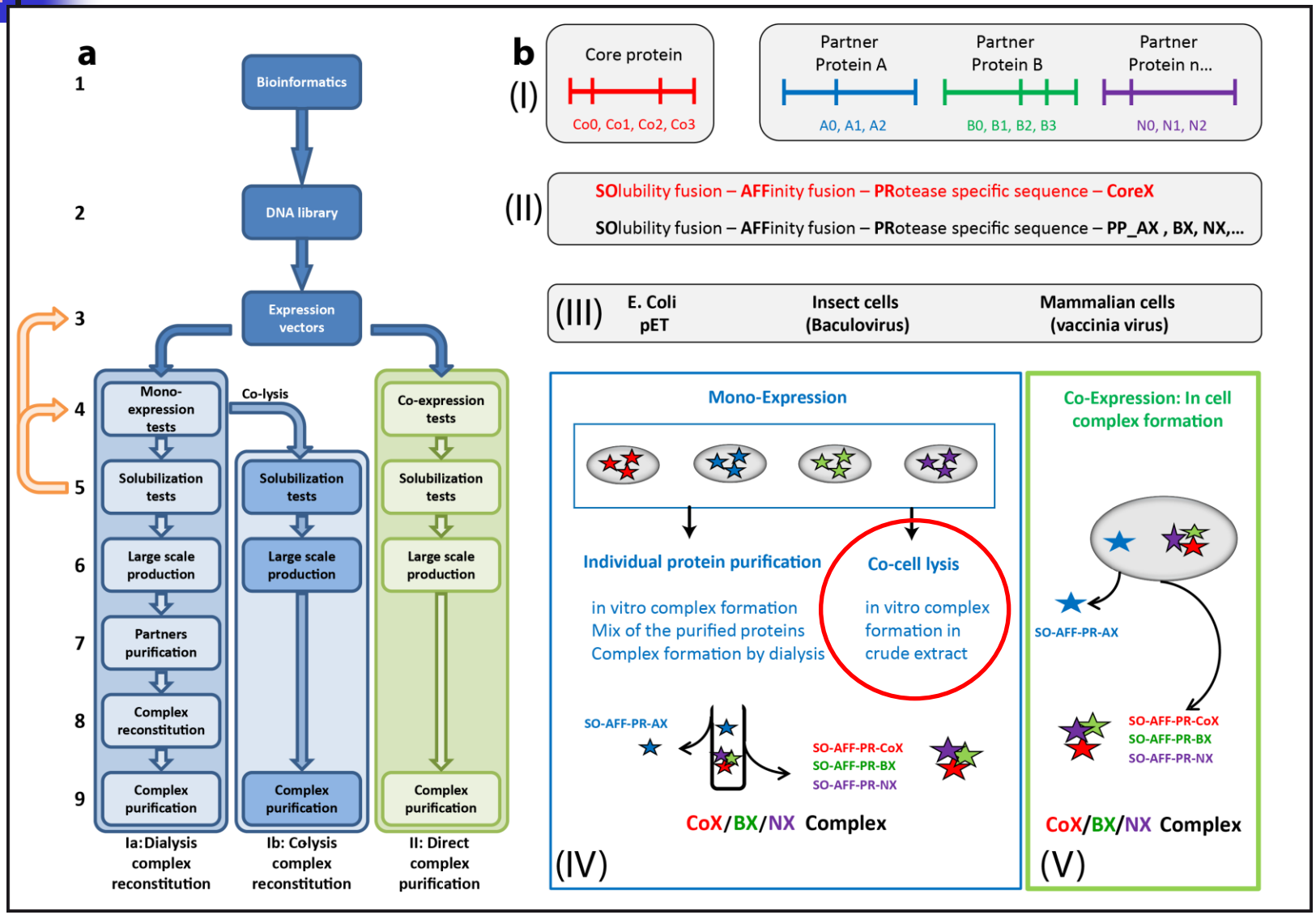
The optimized buffer for protein solubilization is 300 mM KCl, 100 mM Phosphate buffer pH=7.5, 10 mM βmercaptoethanol.

TIF2 Expression and solubilisation tests

TIF2	HIS		GST		MBP	
	LBS/18°C		LBS/18°C		LBS/18°C	
	T	S	T	S	T	S
378-828	+	+/-	+	+	+++	++
378-1008	nd	nd	nd	nd	+++	++
623-772	+	+	+	+	+++	+++
623-828	+	+/-	+	+	+++	++
623-1008	+	+/-	+	+	+++	++

Solubilization buffer: 50mM Phosphate Na/K pH 7.5, 50 mM NaCl

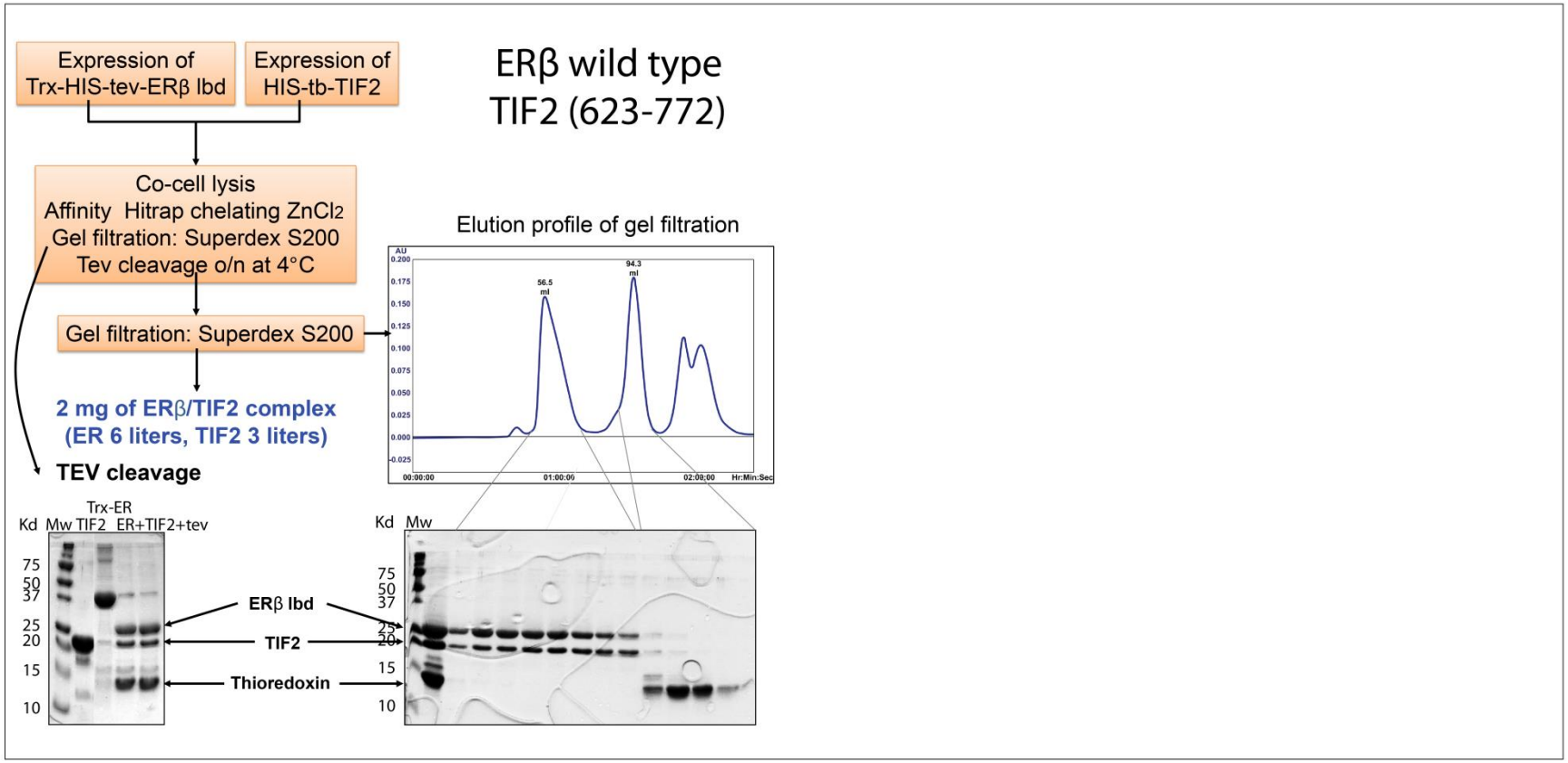
From unstable protein to stable complexes



Levy N, Eiler S, Pradeau-Aubreton K, Maillot B, Stricher F, Ruff M (2016). Nature Communications 7: 10932

ERβ / TIF2 complex reconstitution

Co-cell lysis



TIF2 binds ERβ with an induce folding mechanism

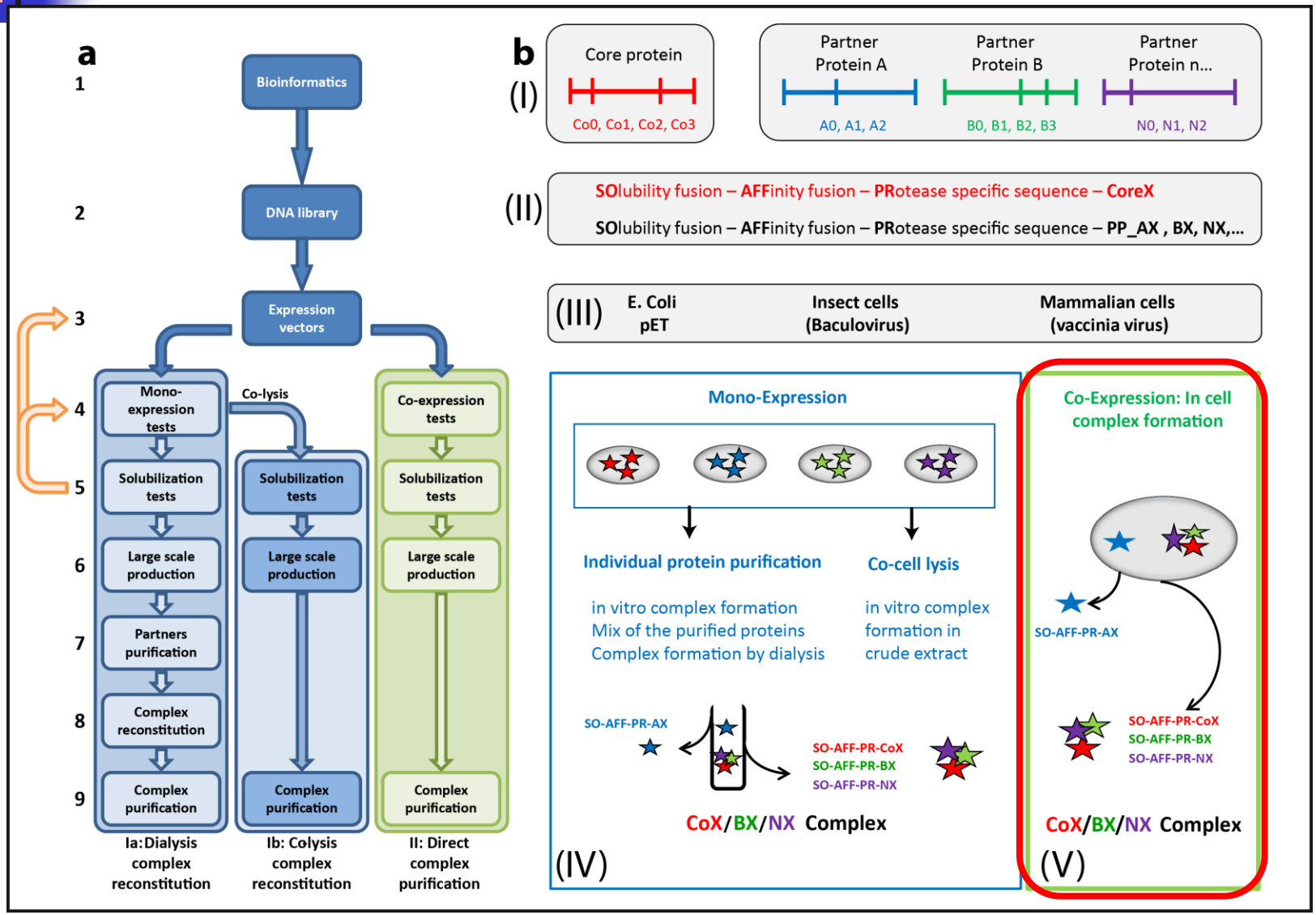
GR Expression and solubilisation tests

GR (*E. coli*)	HIS				FLAG				HIS-NUS			
	LBS/37°C		LBS/18°C		LBS/37°C		LBS/18°C		LBS/37°C		LBS/18°C	
	T	S	T	S	T	S	T	S	T	S	T	S
CDE 420 – 777	+/-	-	-	-	+	-	-	-	++	-	++	+
DE 486 – 777	++	-	-	-	++	-	+	-	++	-	++	++
E 516 – 777	+/-	-	+/-	-	++	-	+	+/-	++	-	++	+
E 524 – 777	-	-	-	-	+	-	++	-	++	-	++	+

GR (insect)	HIS		GST	
	T	S	T	S
CDE 420 – 777	+	-	++	+/-
DE 486 – 777	nd	nd	++	+/-
E 516 – 777	+	-	++	+/-
E 524 – 777	+	-	nd	nd

Solubilization buffer: 250 mM NaCl, 50 mM Tris pH7.5, 10 μM dexamethasone

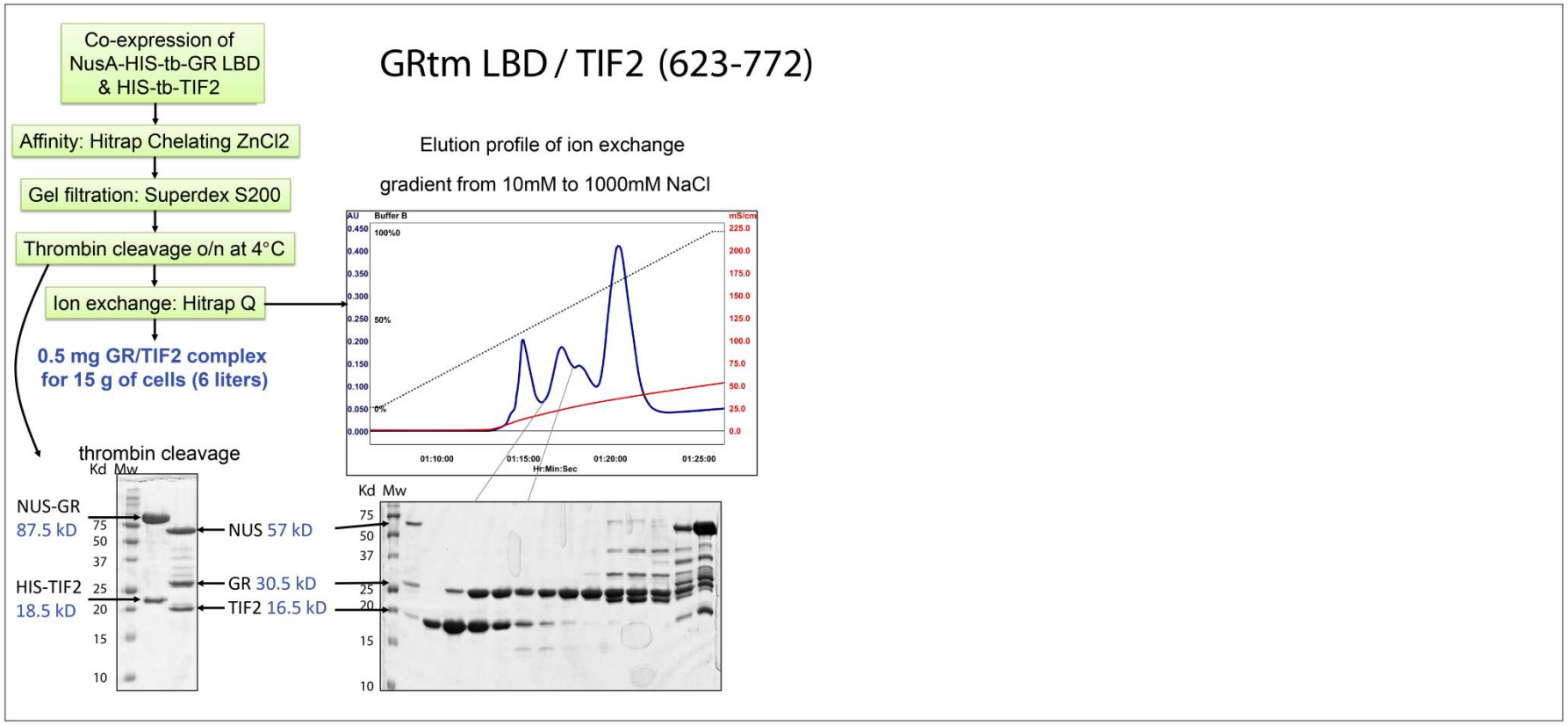
From unstable protein to stable complexes



Levy N, Eiler S, Pradeau-Aubreton K, Maillot B, Stricher F, Ruff M (2016). Nature Communications 7: 10932

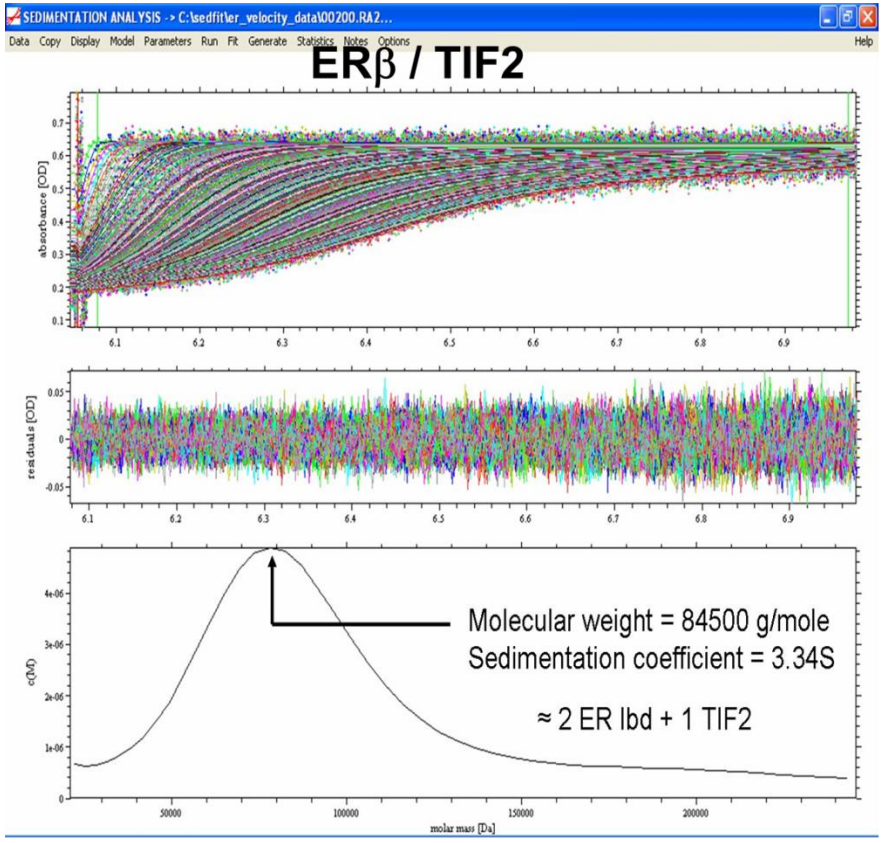
GRtm / TIF2 complex reconstitution

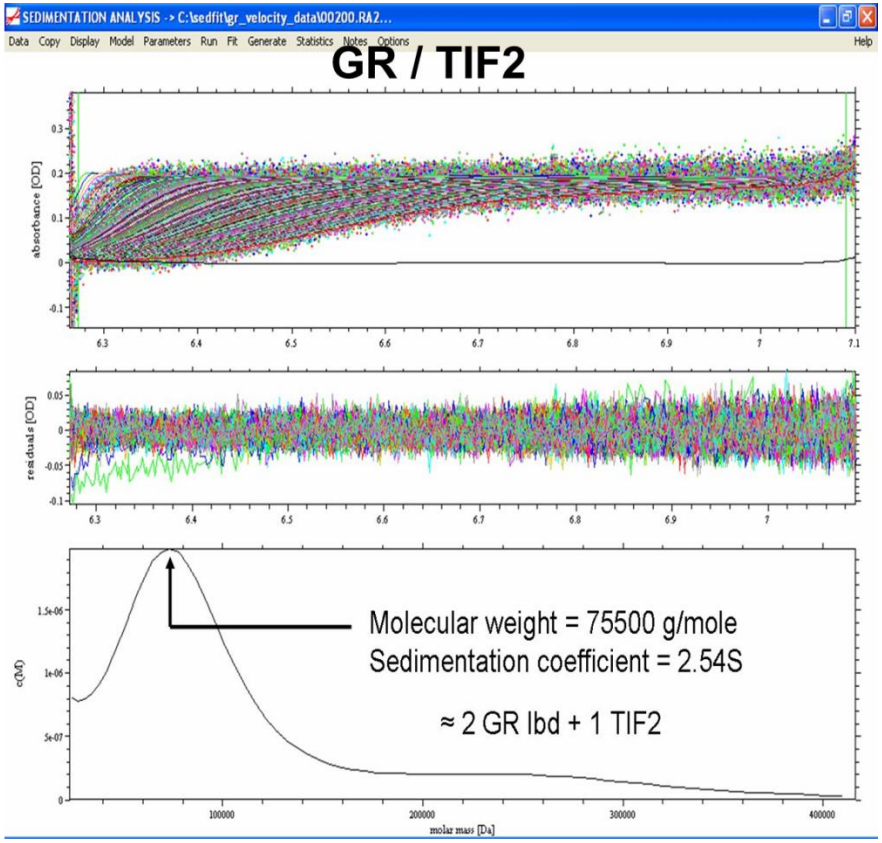
Co-expression



TIF2 binds GR with an induce folding mechanism

Analytical Ultracentrifugation analysis

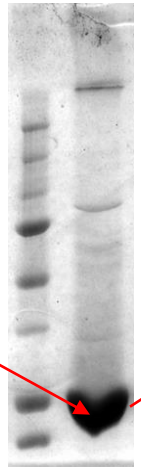




Purification of GR LBD and GR LBD/TIF2

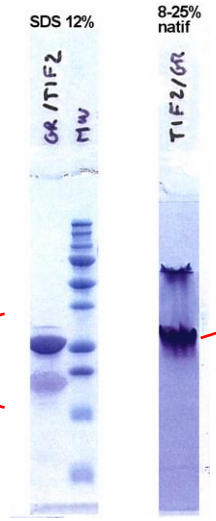
Purification of GR :
High salt
Detergent

SDS PAGE



Crystallization

SDS Natif

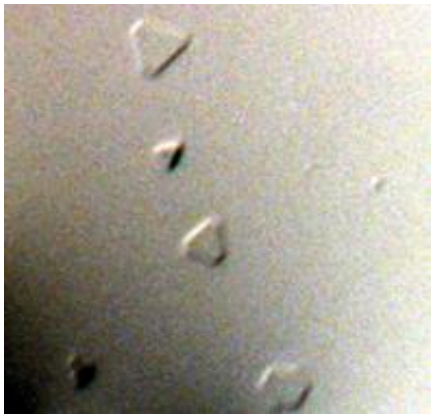


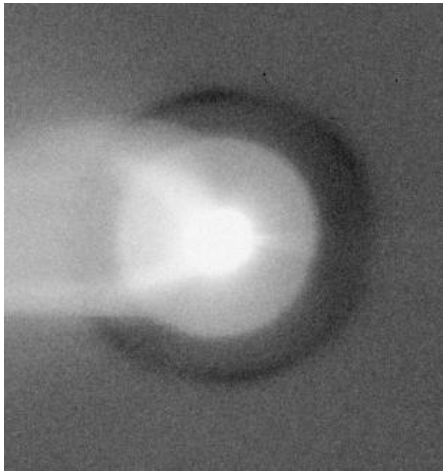
Purification of GR/TIF2 :
Low salt
Without detergent

Mass Spectrometry
Crystallization

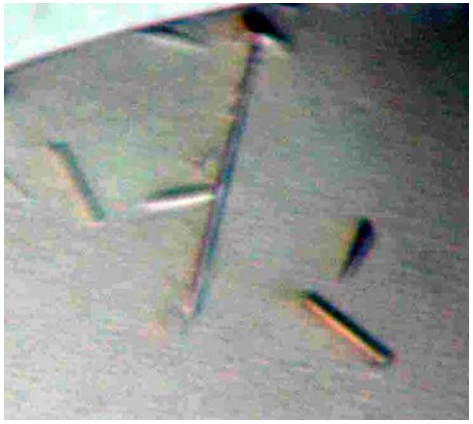
Crystallization of GR LBD + Dex

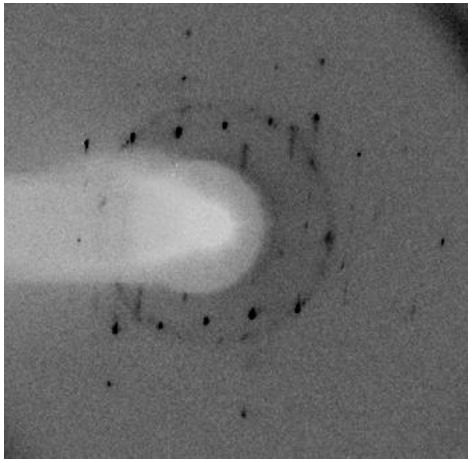
Average size : 5 – 10 μm





Ammonium sulfate 37°C





PEG 1500 37°C

Drug design: structure with ligands (Ligand screening)

Thioredoxin-His-ERαLBD Purification

+ ligand

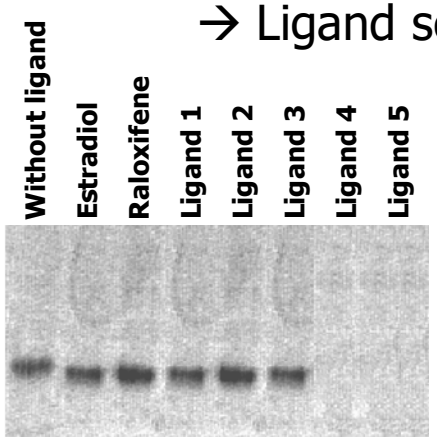
Native Gel

Thrombin cut

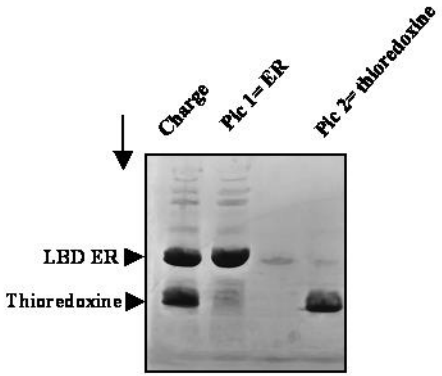
Purification

Crystallization

ERαLBD without ligand not stable
→ Thioredoxin fusion OK
→ Thrombin cut after ligand addition
→ Ligand screening

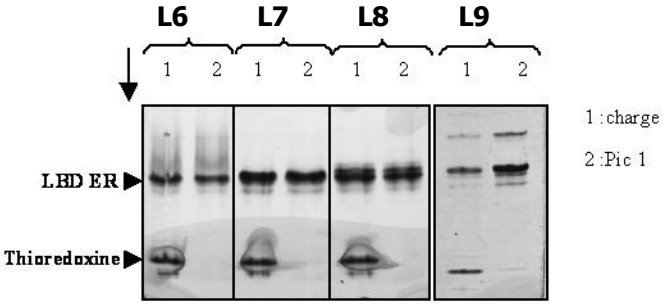


2nd Zinc affinity column



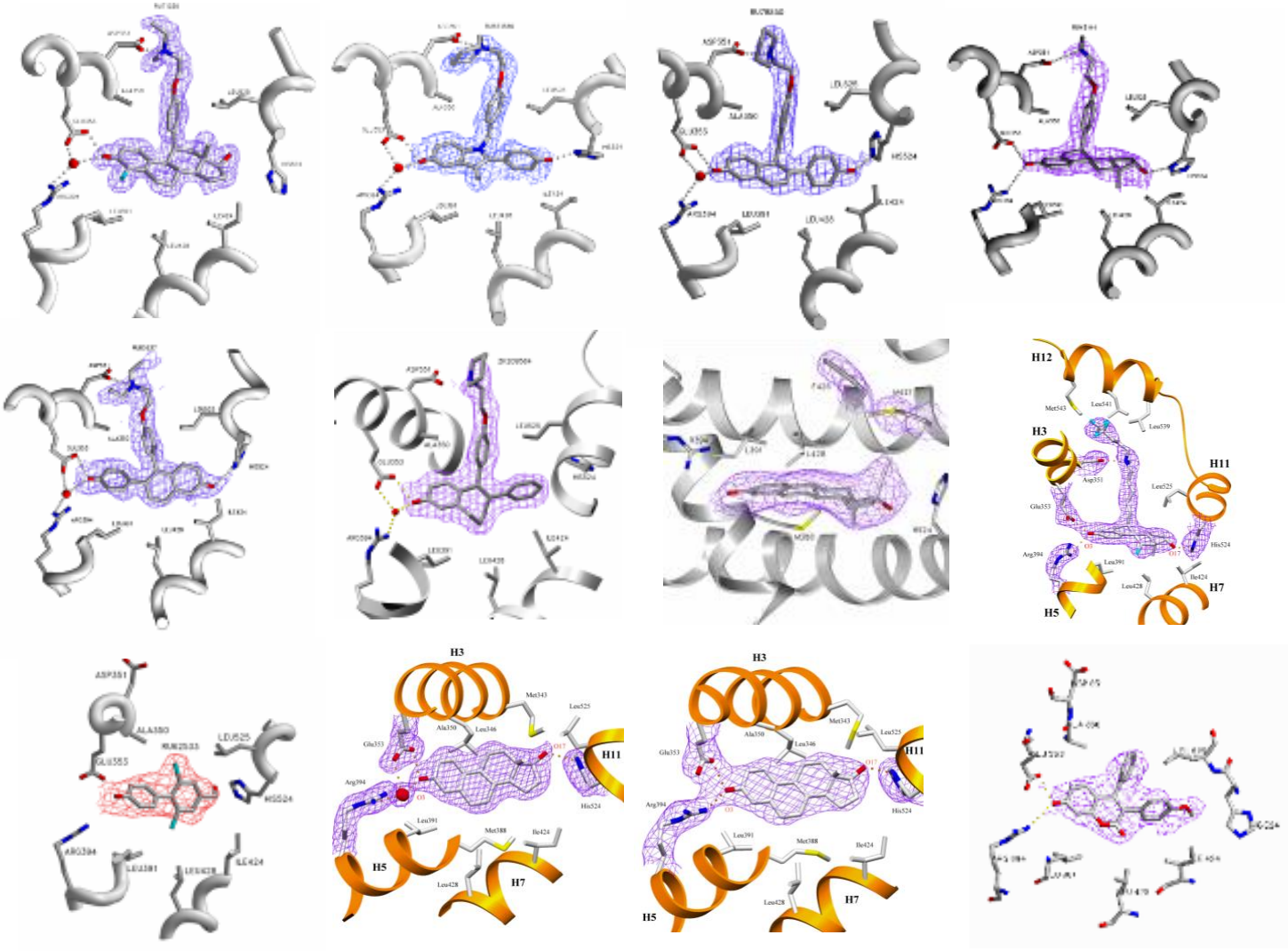
SDS gel with gradient 8-25%

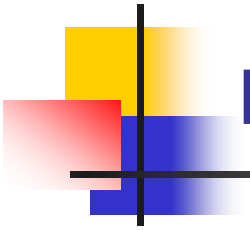
2nd Zinc affinity column



Native gel with gradient 8-25%

ER alpha LBD: ligand screening

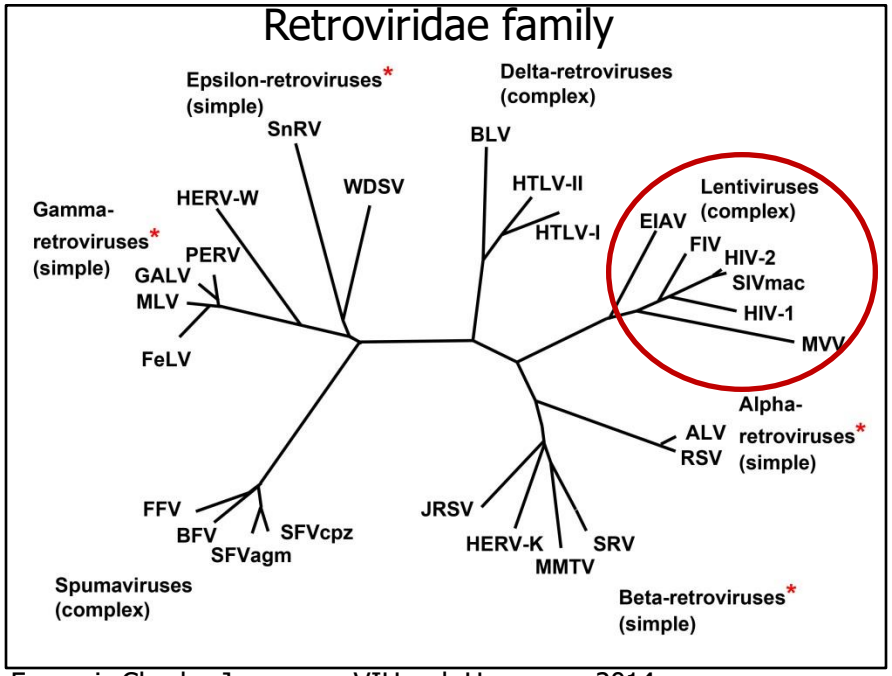


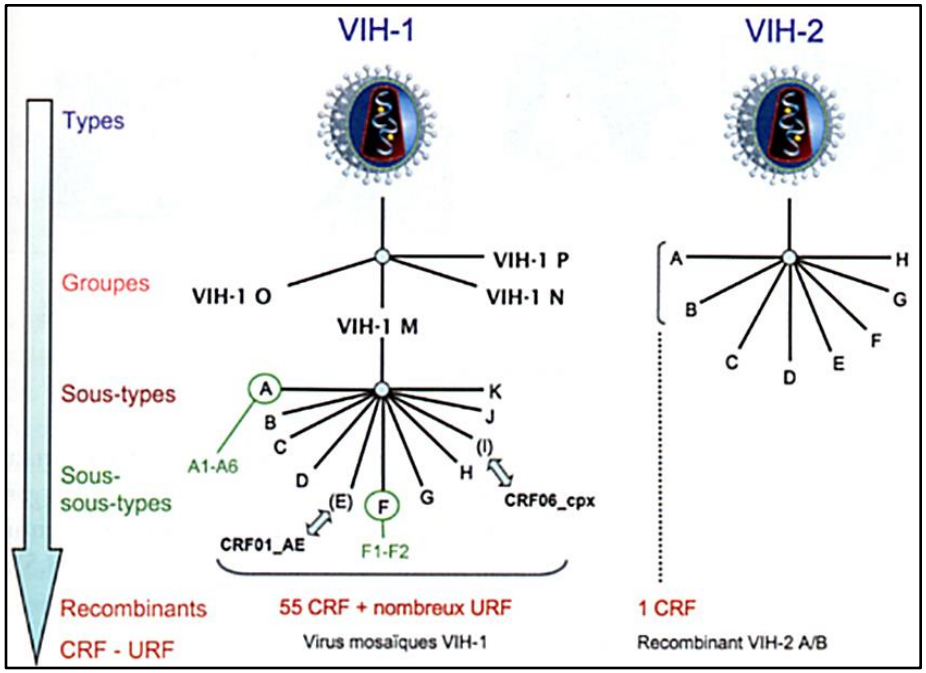


Example 2

HIV-1 Pre-integration complexes

HIV phylogeny





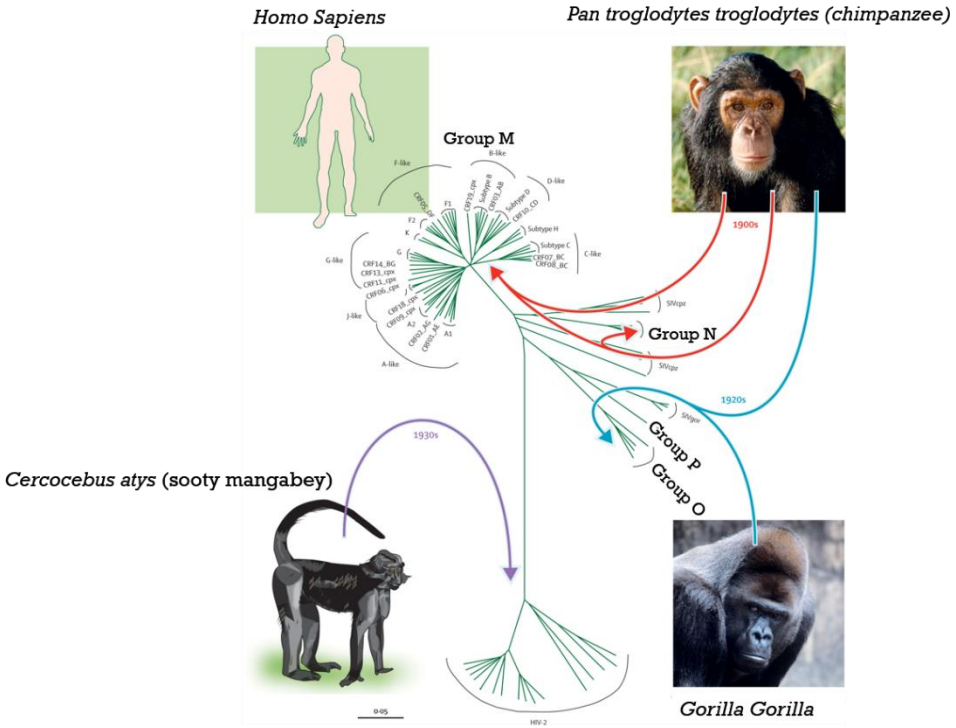
François Charles Javaugue, VIH, ed. Hermann, 2014

HIV-1 and 2 are lentiviruses, a genus of viruses of the Retroviridae family, characterized by a long incubation period.

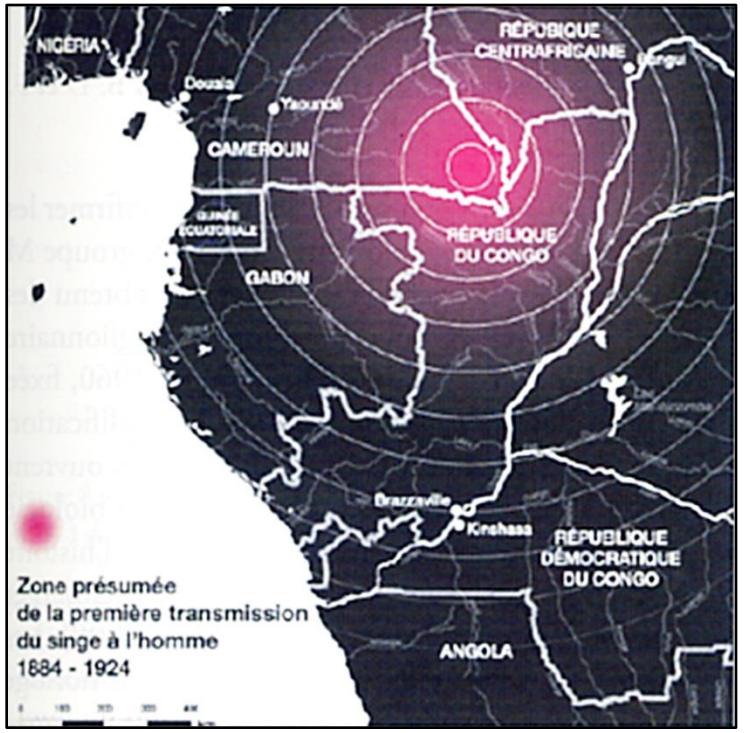
There are many groups and subtypes of HIV-1 virus, the predominant form in Europe is the group M subtype B.

Enveloped virus (budding from the host cell enveloped by fragment of the cell membrane)

Origin of HIV pandemic



Adapted from Tebit *et al.*, Lancet, 2010



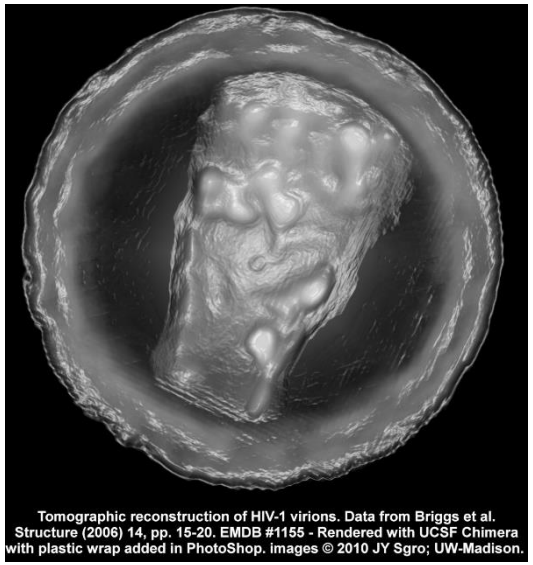
François Charles Javaugue, VIH, ed. Hermann, 2014

HIV emerged from SIV in late 19th / early 20th century through zoonosis between apes or monkeys and men:

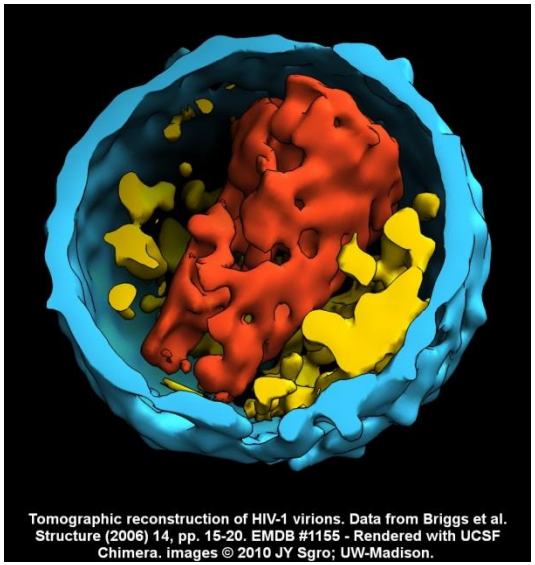
HIV-1 group M : chimpanzee (Central Africa : Cameroon, Equatorial Guinea, Gabon…)
HIV-1 group P : gorilla (Cameroon)
HIV-2 : sooty mangabey monkey (West Africa : Sierra Leone, Liberia, Ivory Coast…)

HIV-1 virion structure

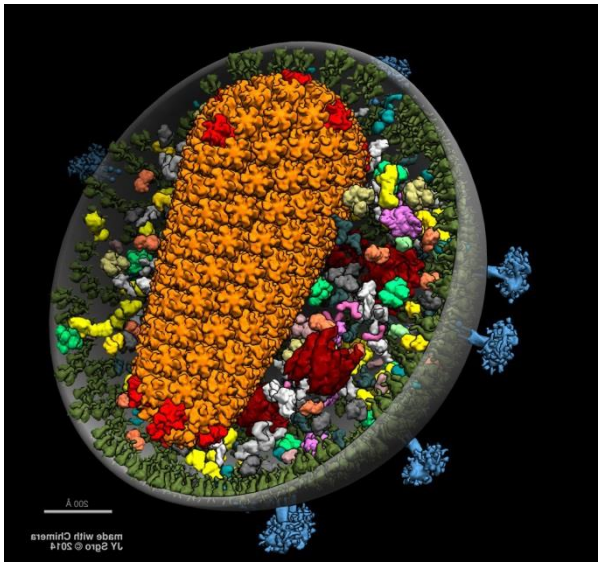
JY Sgro, http://www.virology.wisc.edu/virusworld, 2009

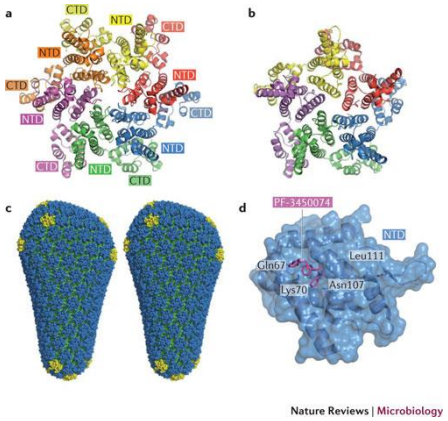


Tomographic reconstruction of HIV-1 virions. Data from Briggs et al. Structure (2006) 14, pp. 15-20. EMDB #1155 - Rendered with UCSF Chimera with plastic wrap added in PhotoShop. images © 2010 JY Sgro; UW-Madison.

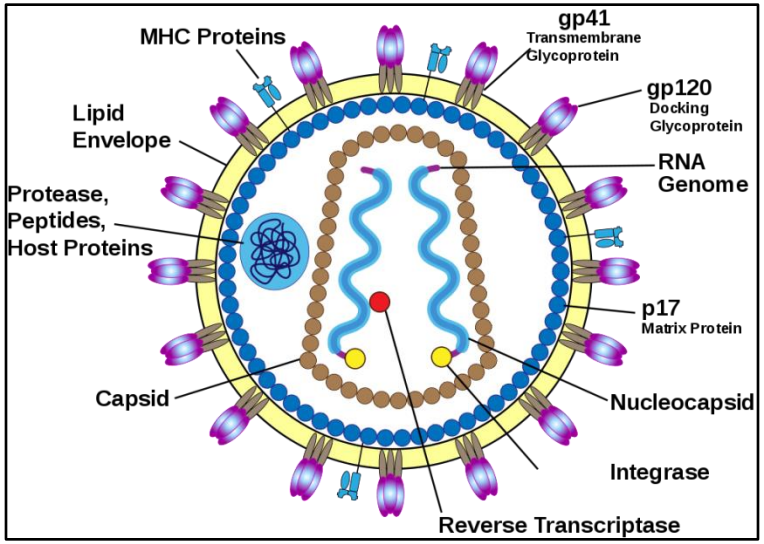


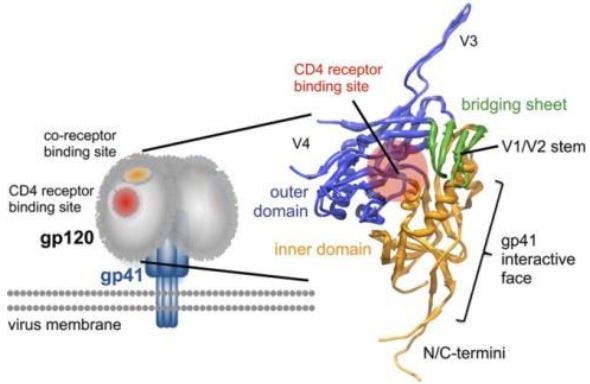
Tomographic reconstruction of HIV-1 virions. Data from Briggs et al. Structure (2006) 14, pp. 15-20. EMDB #1155 - Rendered with UCSF Chimera. images © 2010 JY Sgro; UW-Madison.





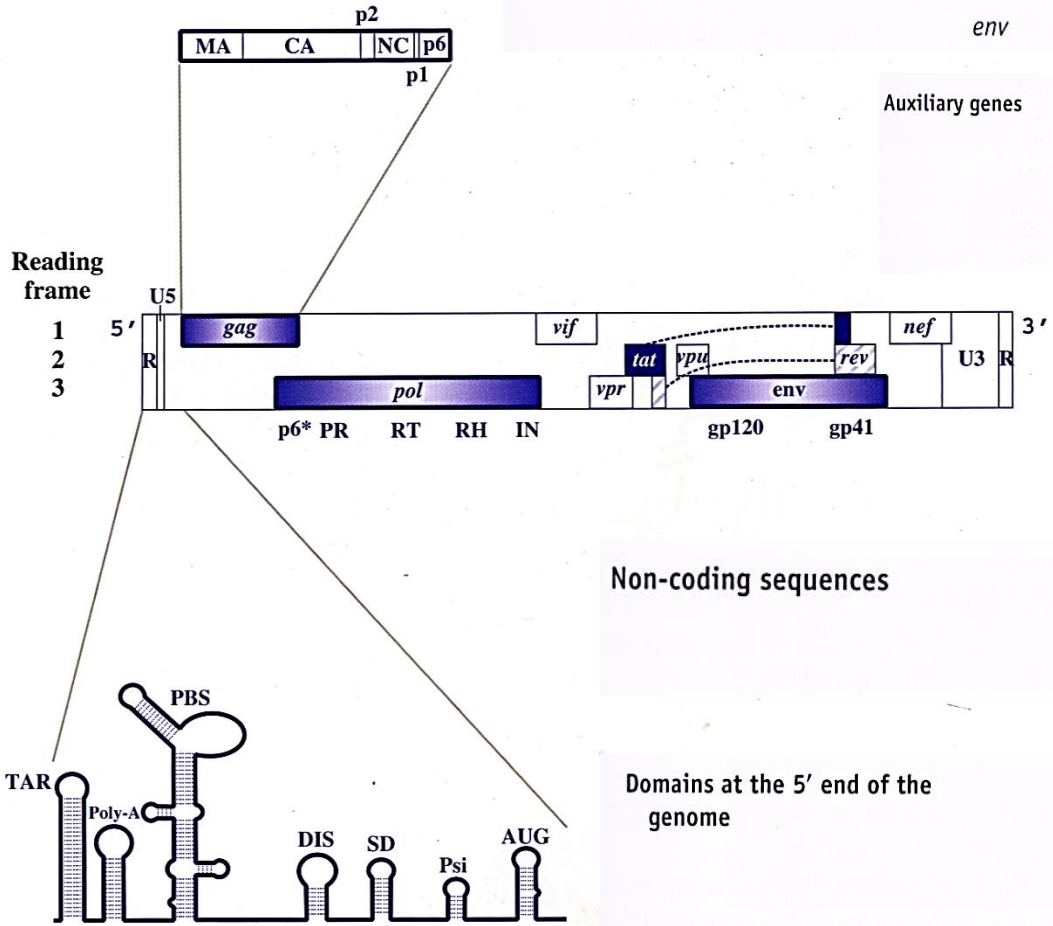
Alan Engelman and Peter Cherepanov, Nature Review in Microbiology, 2012, 10, 279-289





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	*r*egulator 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*

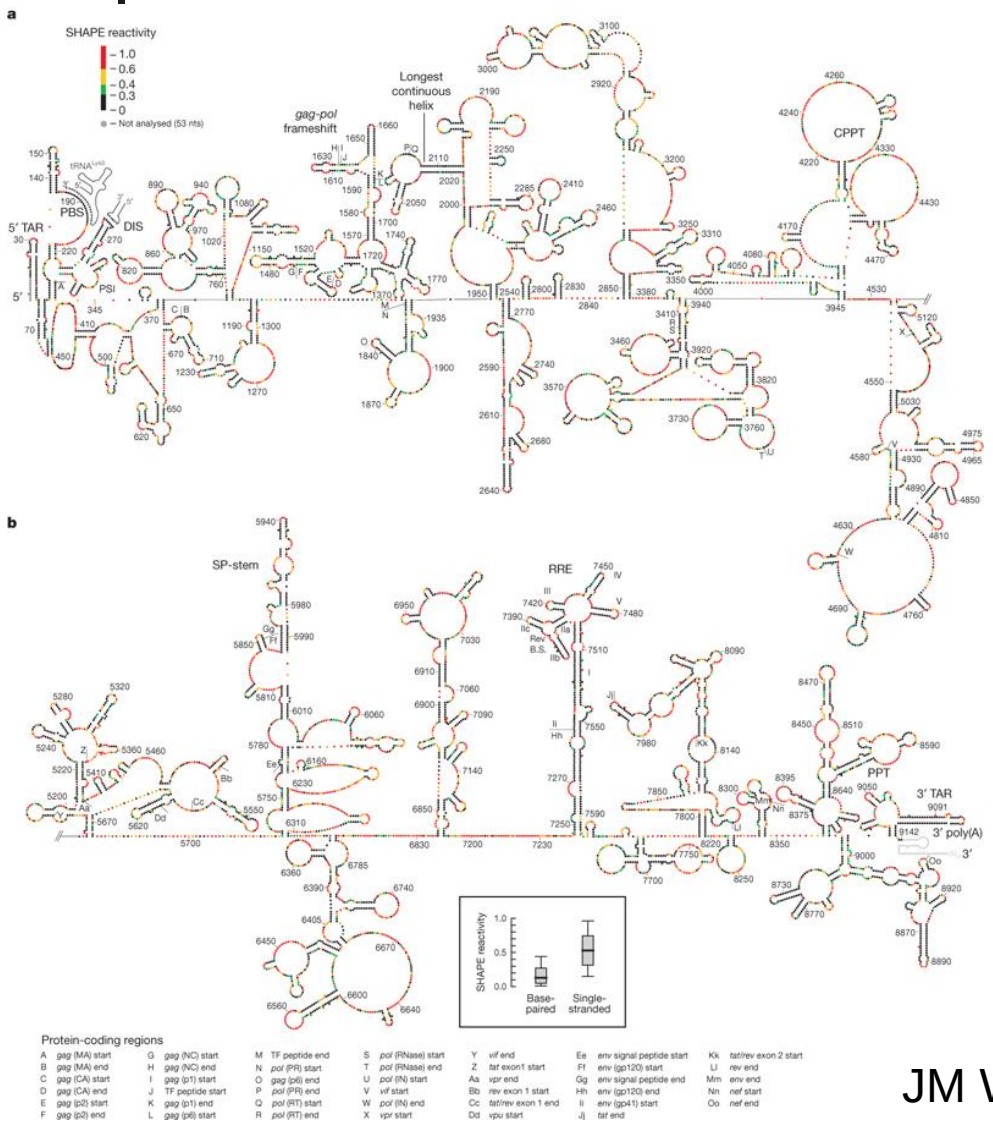
Non-coding sequences

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

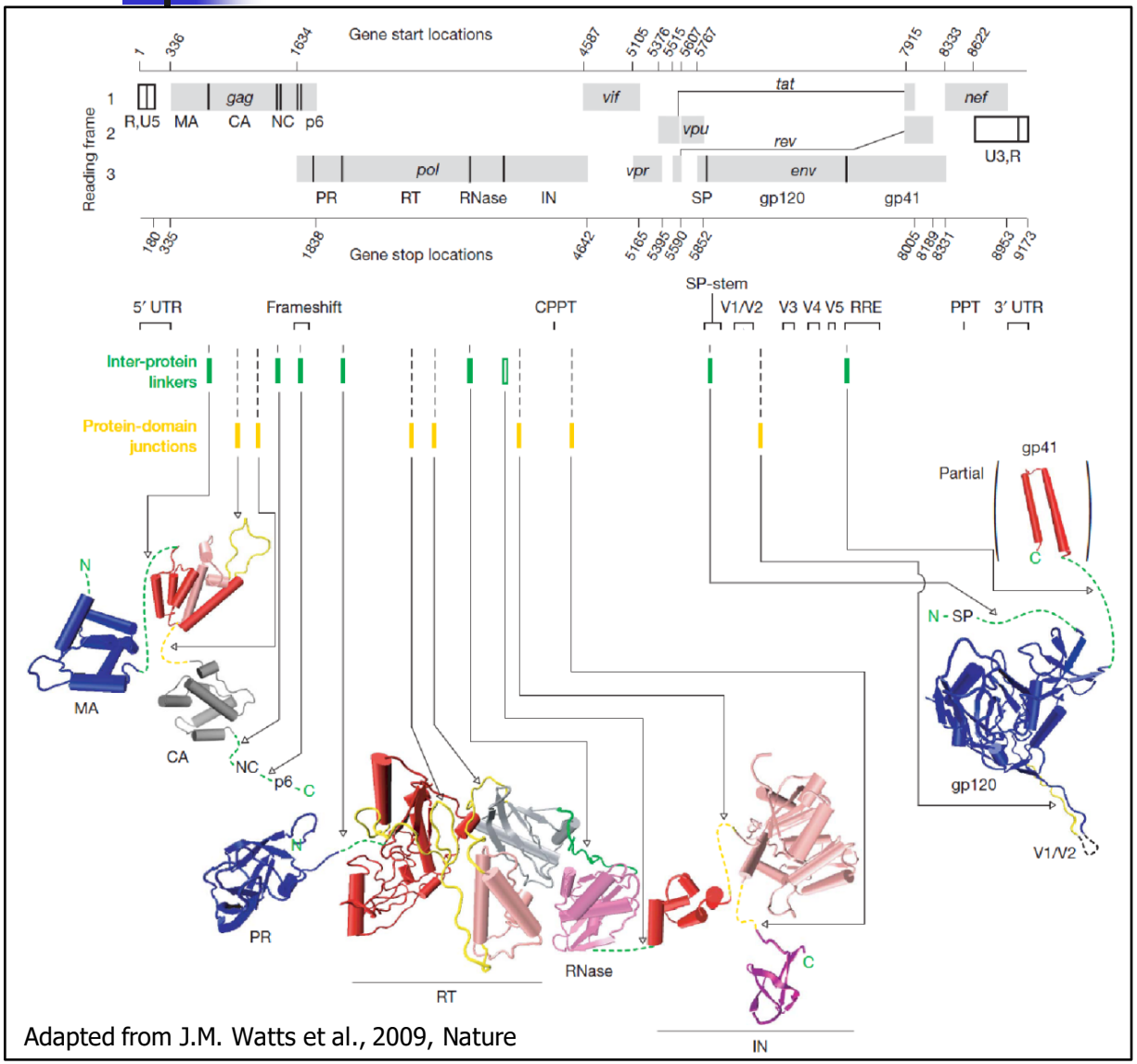


The 5' (**a**) and 3' (**b**) genome halves are shown. Nucleotides are coloured by their absolute SHAPE reactivities. Every nucleotide is shown explicitly as a sphere; base pairing is indicated by adjacent parallel orientation of the spheres.

Intermolecular base pairs involving the $tRNA^{Lys3}$ primer and the genomic dimer are shown in grey.

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

HIV genome and viral protein structures



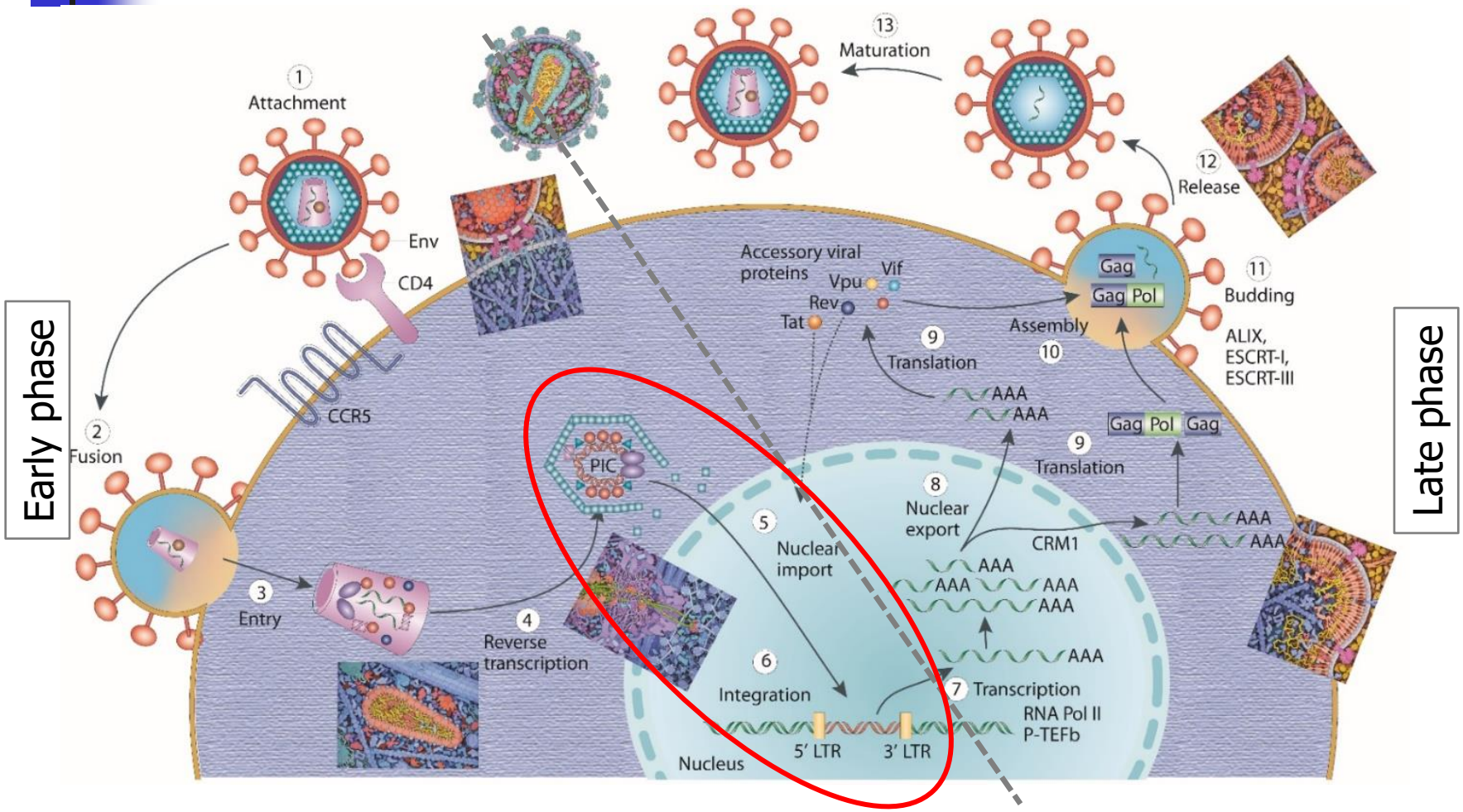
Adapted from J.M. Watts et al., 2009, Nature

3 main genes coding for the viral polyproteins :
Gag, **Pol** and **Env**.
Gag => structural proteins
Pol => viral enzymes
Env => envelope proteins

2 regulatory genes:
Tat and **Rev** (activation of transcription and regulation of RNA splicing and export)

Accessory genes :
Vif, Vpr, Vpu, Nef
(regulation of synthesis and processing viral RNA and other functions)

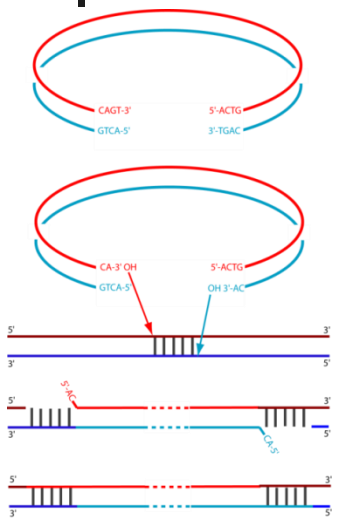
Schematic diagram of HIV replication cycle

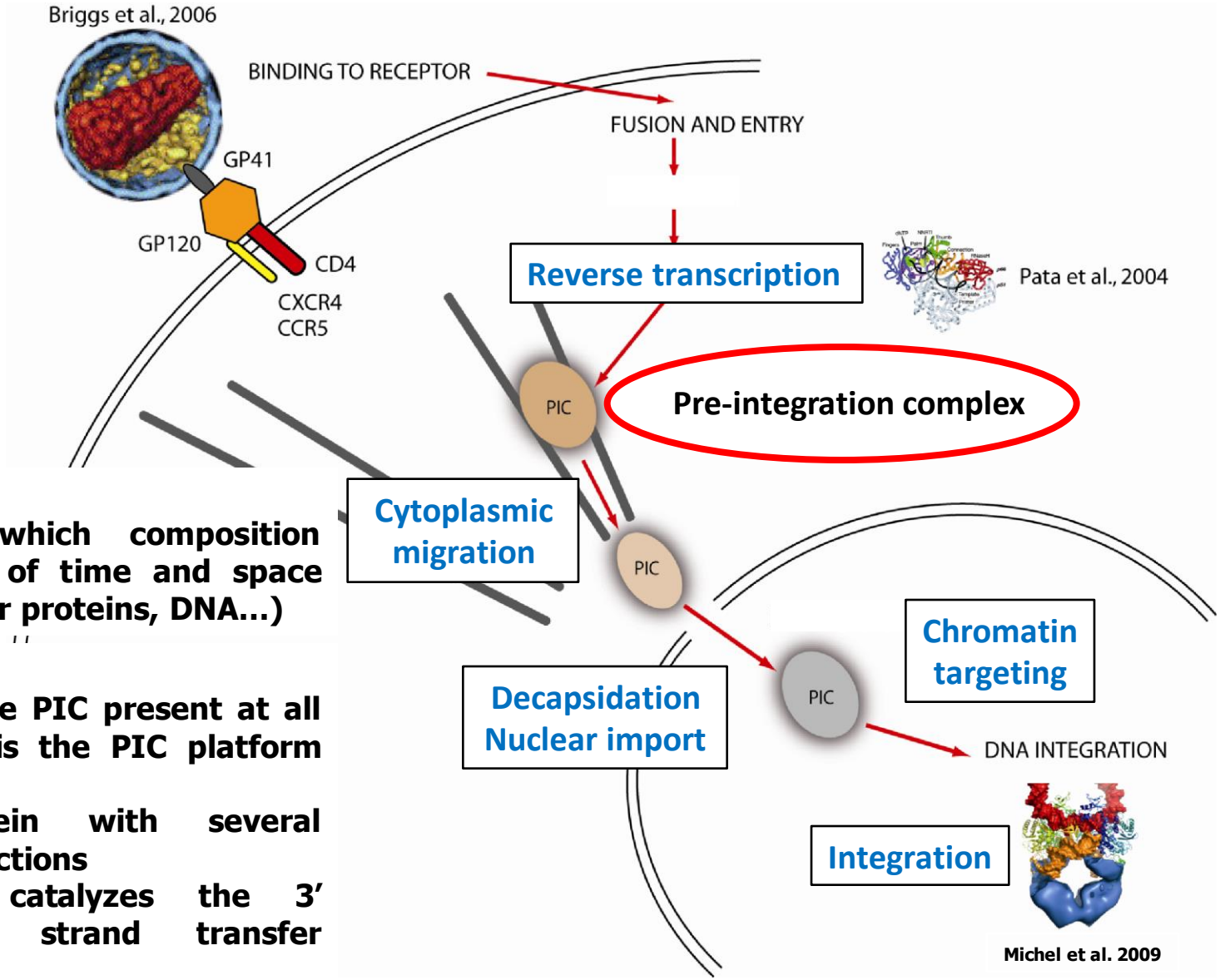


Adapted from Engelman et al., 2012

Constant need for new generations of inhibitors in AIDS treatment :
Need of precise knowledge of replication mechanisms

HIV-1 pre-integration complex





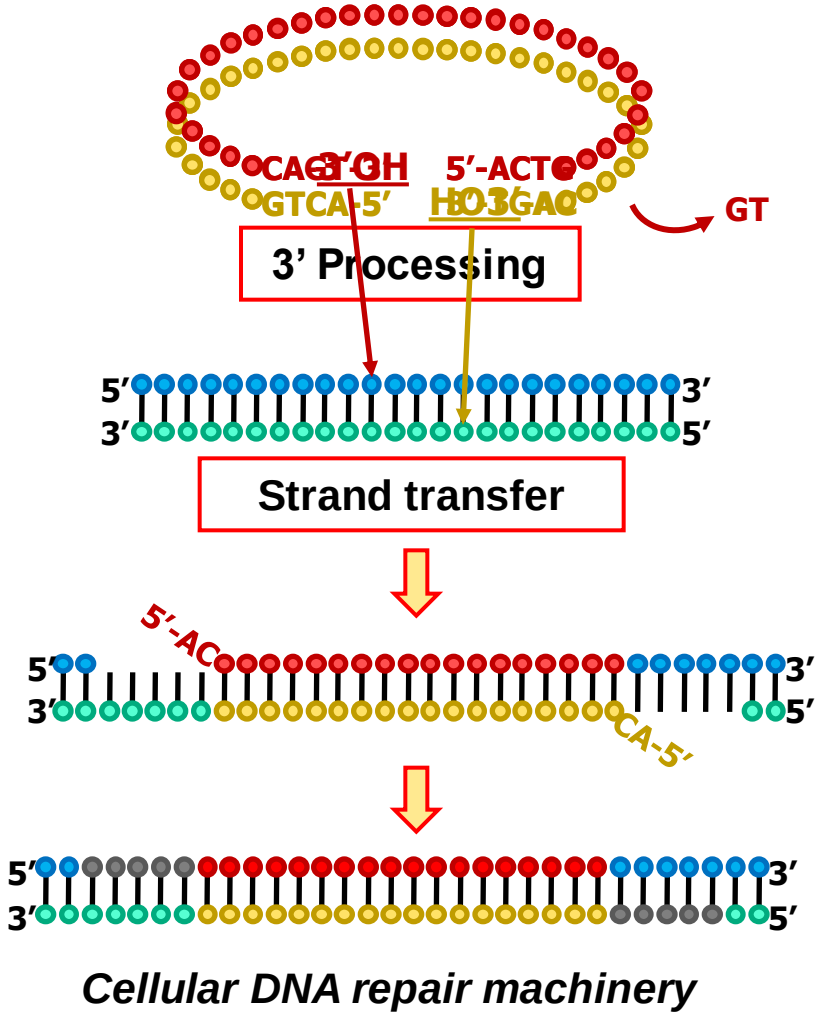
PIC:

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**

Catalytic activities of HIV-1 integrase



Viral cDNA

Host cell target DNA

Integrated proviral DNA

The HIV-1 viral DNA integration

David S. Goodsell, http://hive.scripps.edu/resources.html

Decapsidation
Reverse transcription

Nuclear import

Continuous and dynamic process

Integration

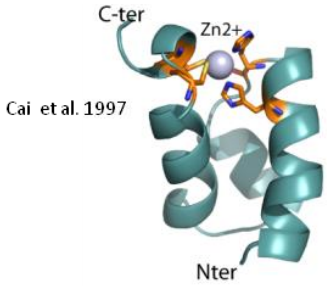
Structural domains of HIV-1 integrase

HIV integrases family

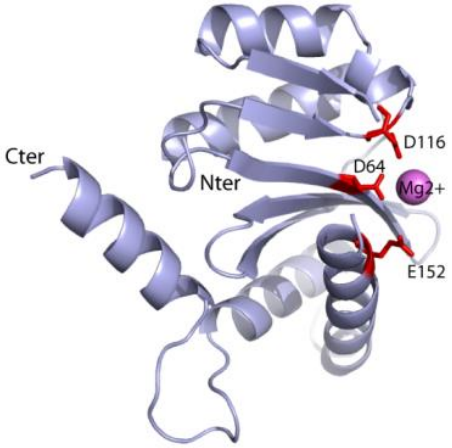
Ribonucleases H superfamily
Interaction with LEDGF

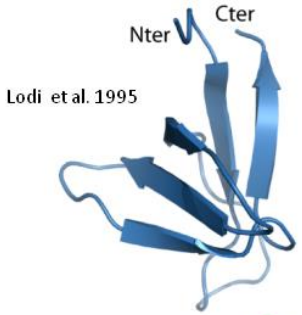
Retroviral integrases superfamily

N-terminal domain (NTD) | Catalytic Core Domain (CCD) | C-terminal domain (CTD)

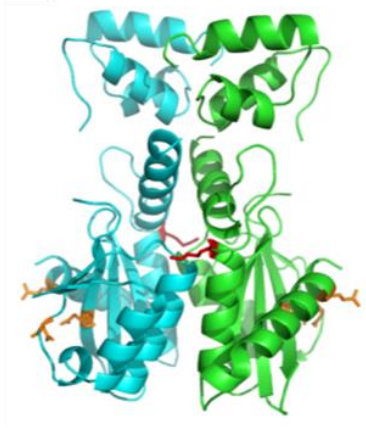


Cai et al. 1997

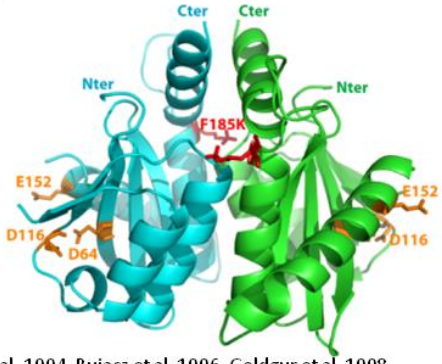




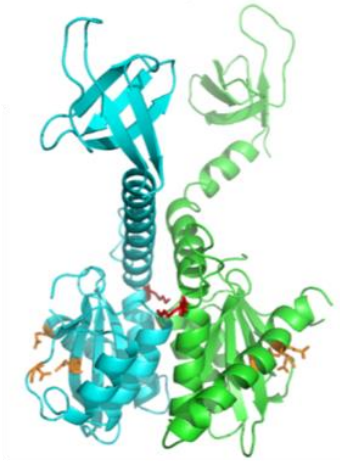
Lodi et al. 1995



Wang et al. 2001

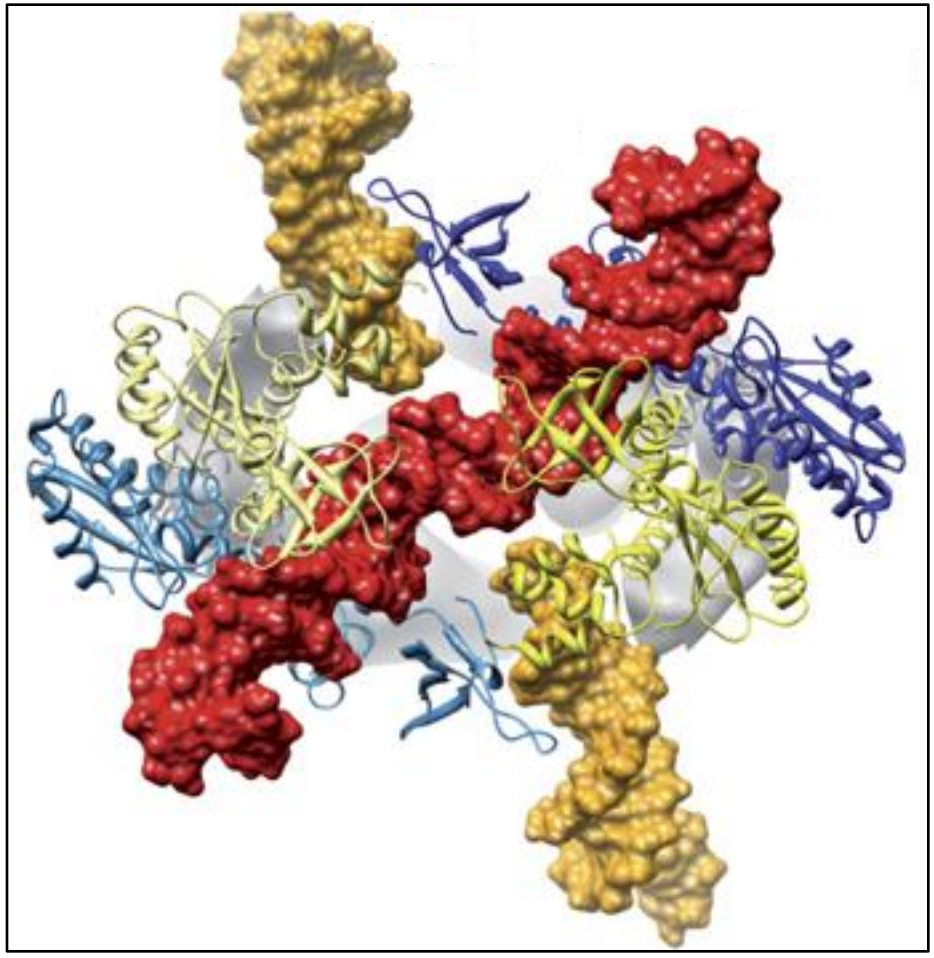


Dyda et al. 1994, Bujacz et al. 1996, Goldgur et al. 1998
Maignan et al. 1998, Greenwald et al. 1999, Chen et al. 2000

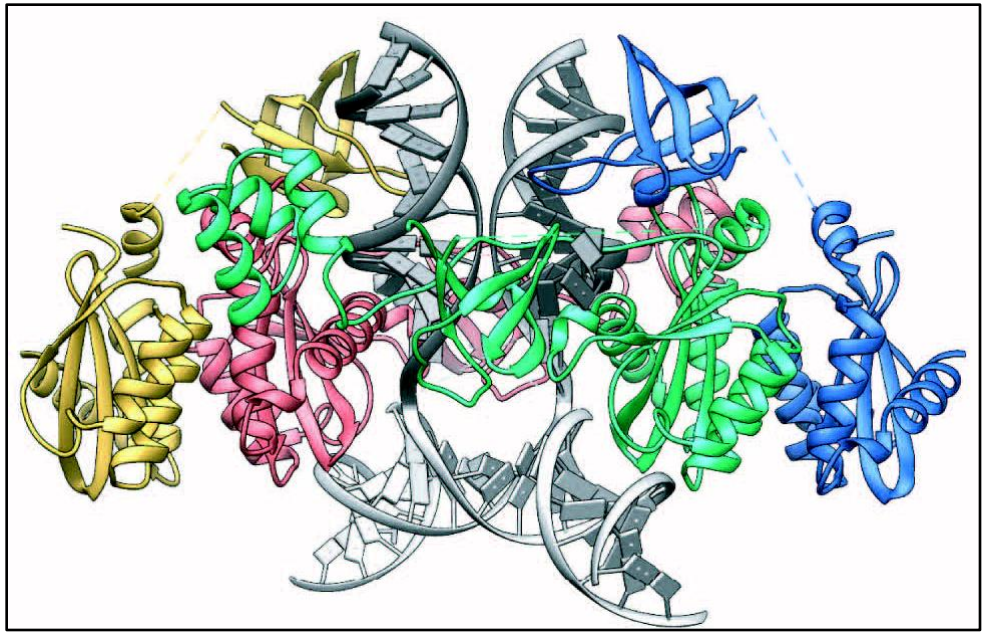


Chen et al. 2000

Comparison of HIV-1 integrase complexes

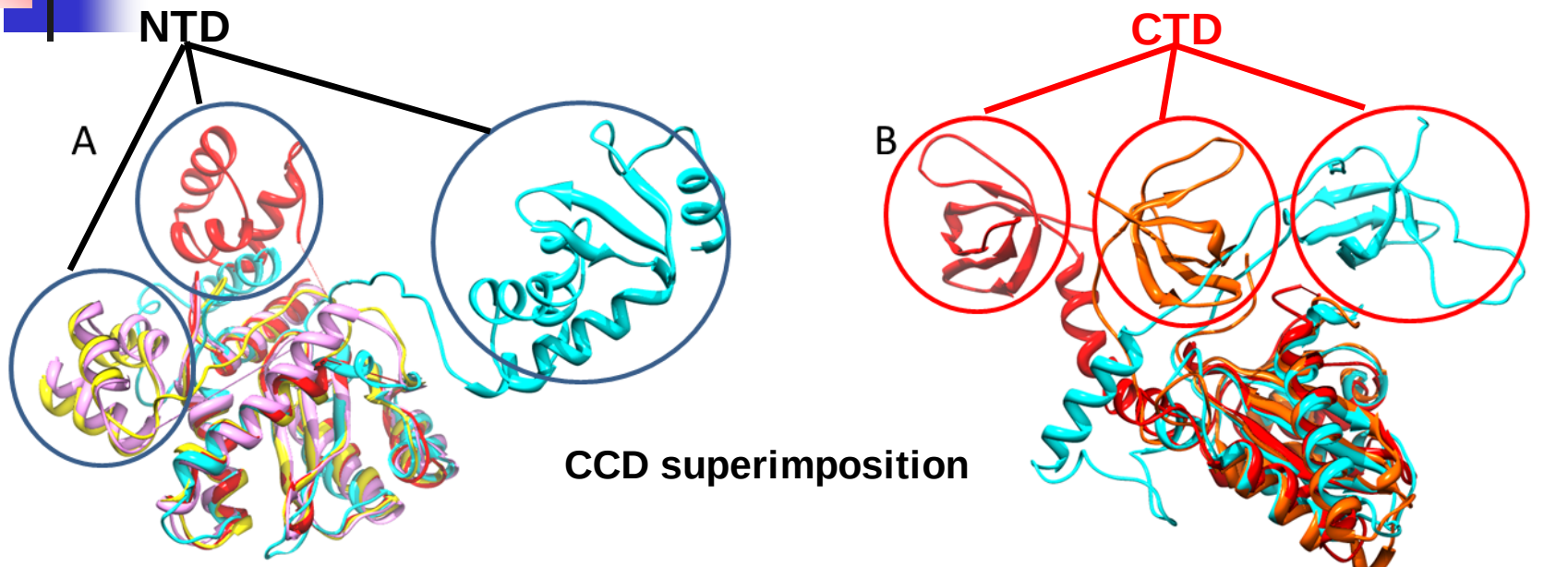


Adapted from Michel *et al.*, EMBO 2009



Adapted from Passos *et al.*, Science 2017

HIV Integrase



A. Superimposition of NTD+CCD structures:
MVV (Hare et al., 2009a), PFV (Hare et al., 2010),
HIV-2 (Hare et al., 2009b) , HIV-1 (Wang et al., 2001)

B. Superimposition of CCD+CTD structures :
RSV (Yang et al., 2000), HIV-1 (Chen et al., 2000)
PVF (Hare et al., 2010).

High flexibility allows to accommodate different partners and functions
No high resolution structure of full-length HIV integrase
=> Stabilization of integrase with partners/ligands for structural and functional studies

Structural and functional studies

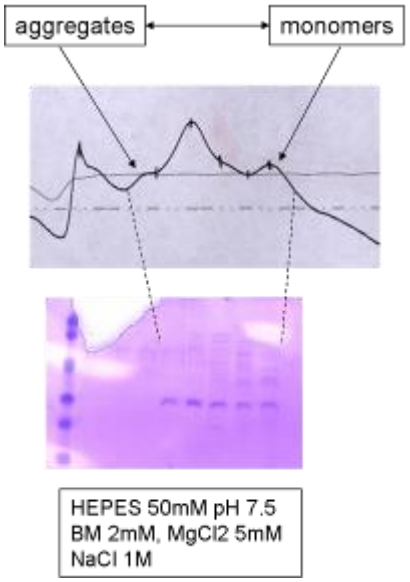
Isolate stable IN complexes (VBP1, TRN-SR2, LEDGF, INI1….)

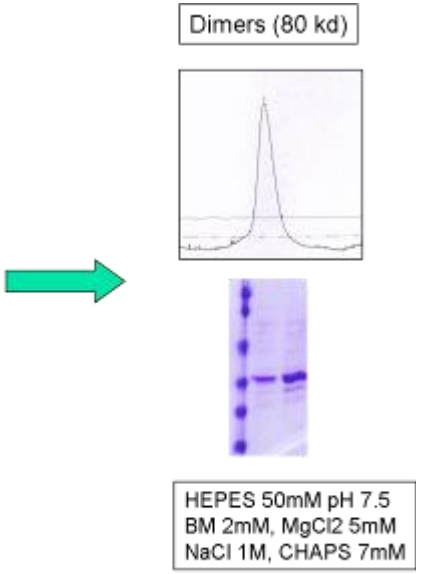
Isolate protein with PTMs:
Production in eukaryotic cells
Specific modifications

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	+	++	+++	+++	++

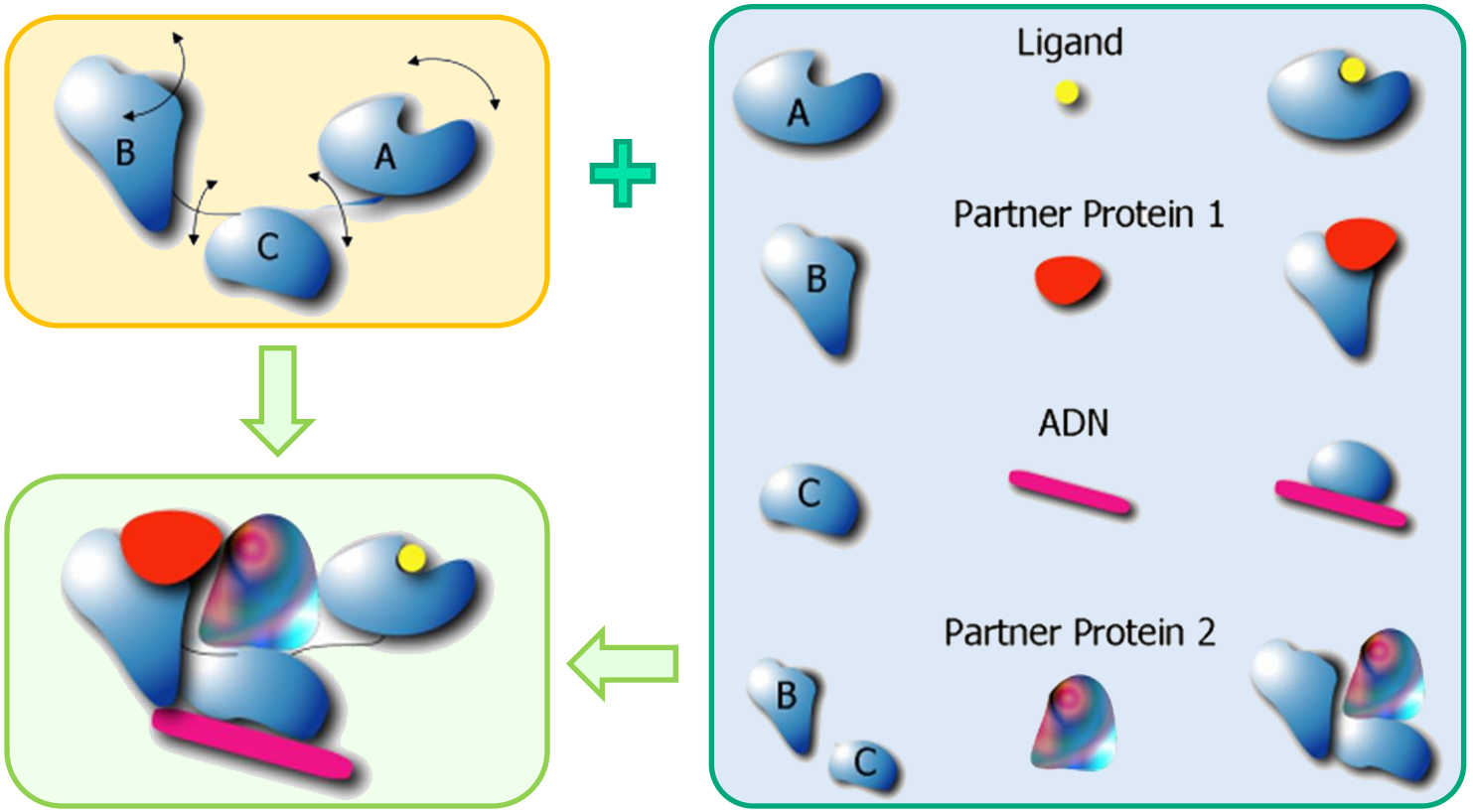




No crystals

Strategy

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



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

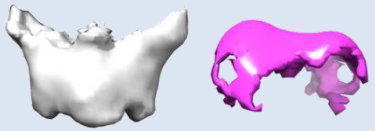
HIV Integrase structural and functional studies

DNAs

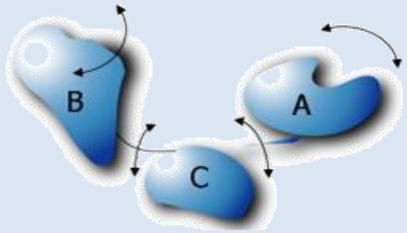
Viral cofactors

vDNA, RT, CA, NC, Ma, Vpr, Rev …

Cellular cofactors



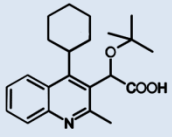
VBP1, TRN-SR, GCN5, P300…

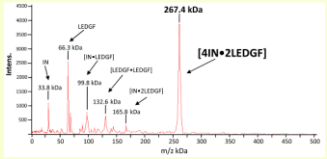


HIV integrase

Post-translational modifications

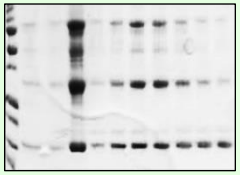
Small molecules



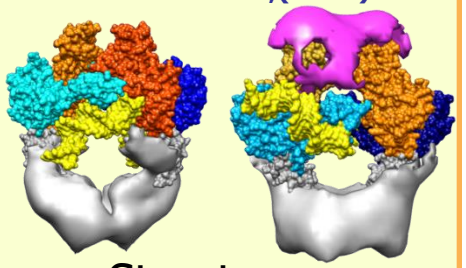


Mass spectrometry

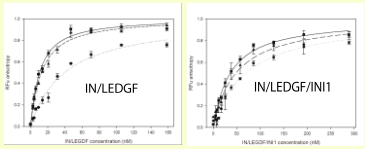
Stable complexes



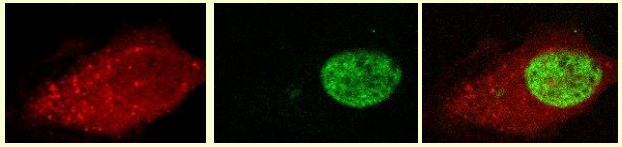
Maillot B. et al,(2013)
Michel F. et al,(2009)



Structures (Xray, CryoEM)



Functional validation *in vitro*



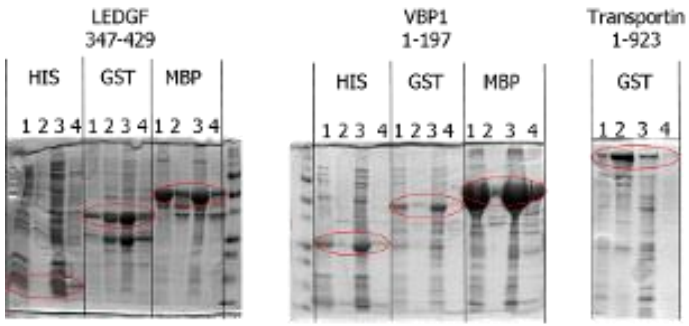
Functional validation *in vivo*
Complexes in the cellular context

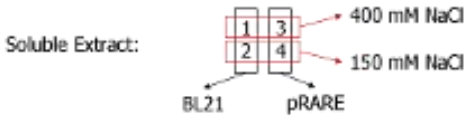
Cloning, expression and solubility tests (E. Coli)

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)

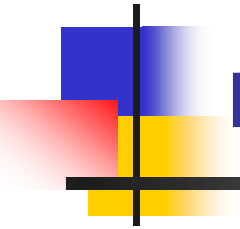
Protein	Fragment	Tag N_{term}	Protease site	Antibiotic	Expression	Solubility HS	Solubility LS	Notes
VBP1	1-197	6xHis	P3C	Amp	++	+++	Nd	
		GST			+++	+++		
		MBP			+++	+++		
		6xHis-MBP			+++	+++		
		6xHis	Tev		+++	+++		
		GST			+++	+++		
		6xHis-MBP			+++	+++		
		6xHis	P3C	spec	Nd	Nd		Bi-cistronic
		0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





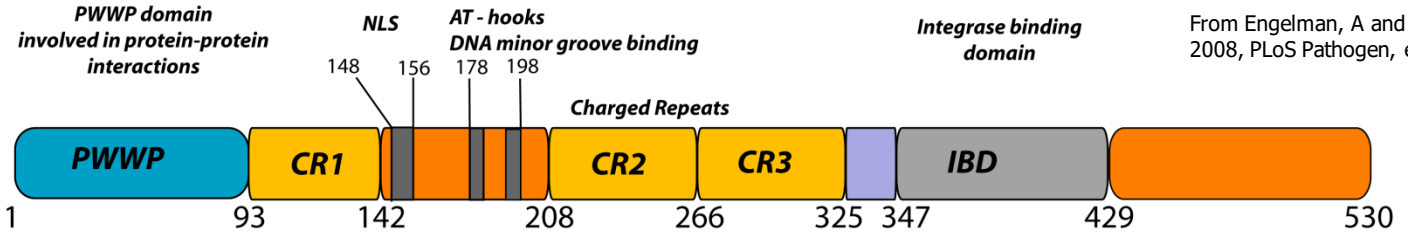
	37°		25°		18°	
	ET	EB	ET	EB	ET	EB
LB	+	-	+	+	+	+
LB/sucrose	+	-	+	+	++	++

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



HIV-1 IN / LEDGF complex

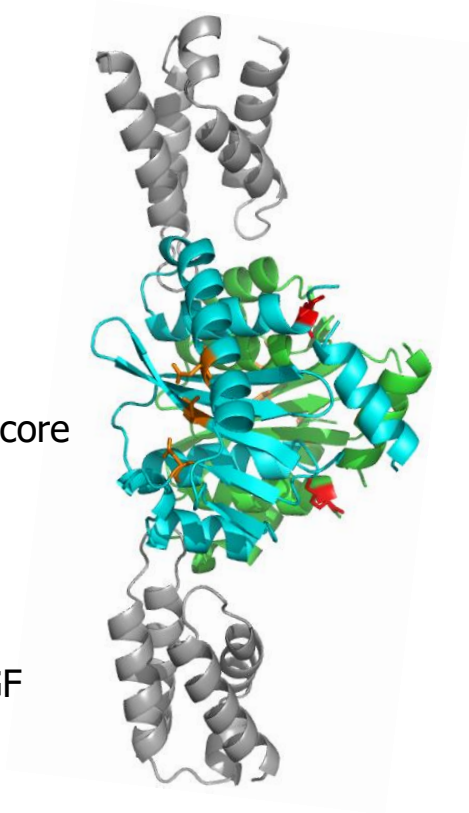
HIV-1 IN interact with LEDGF



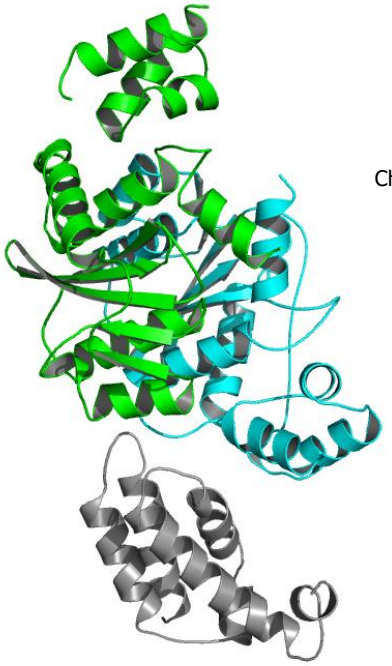
From Engelman, A and Cherepanov, P 2008, PLoS Pathogen, e1000046

IN catalytic core

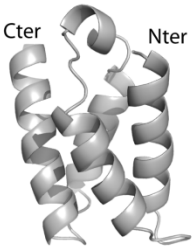
IBD LEDGF



Cherepanov et al. 2005



Hare et al. 2009



Cherepanov et al. 2005

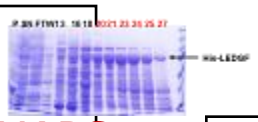
LEDGF/P75

- PC4 and SFRS1 interacting protein (Lens epithelium-derived growth factor) (Transcriptional coactivator p75/p52) (Dense fine speckles 70 kDa protein) (DFS 70) (CLL-associated antigen KW-7).
- Transcriptional coactivator involved in neuroepithelial stem cell differentiation and neurogenesis. Involved in particular in lens epithelial cell gene regulation and stress responses. May play an important role in lens epithelial to fiber cell terminal differentiation. May play a protective role during stress-induced apoptosis.
- Length: 530 aa, molecular weight: 60103 Da, pI = 9.85

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

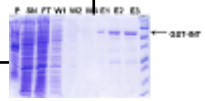
Purification GST-P3C-INT and HIS-Thr-LEDGF

Purification His-Thr-LEDGF



1M NaCl, 7mM CHAPS

Purification GST-P3C-INT



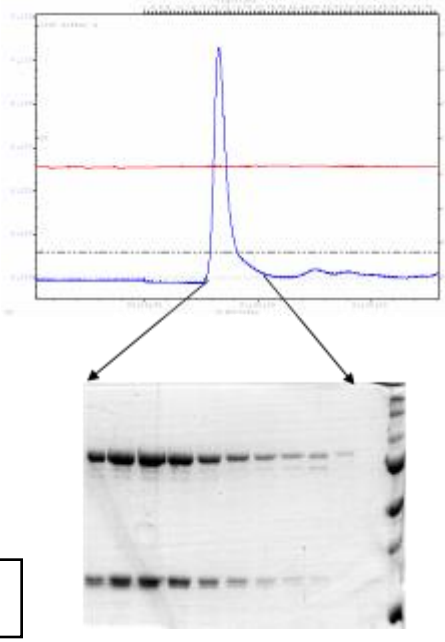
Complex formation by dialysis

GST Affinity

P3C cut

GST Affinity

Gel filtration G200



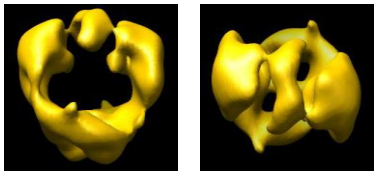
Functional and biochemical characterization

HTS Screening of inhibitors

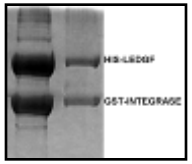
Crystallization Assays

SAXS, SANS studies

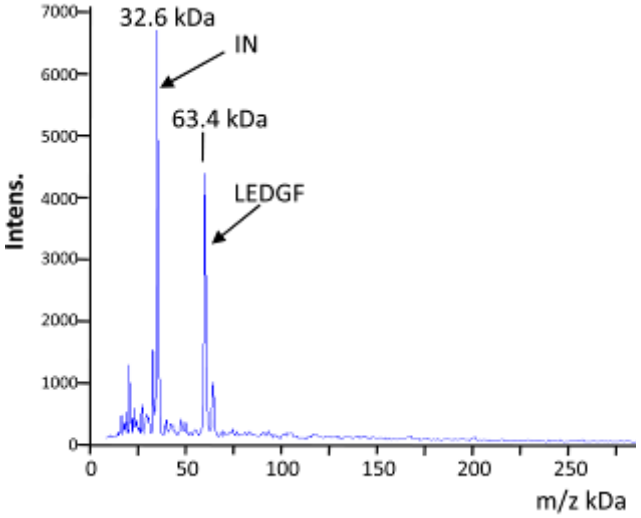
EM studies



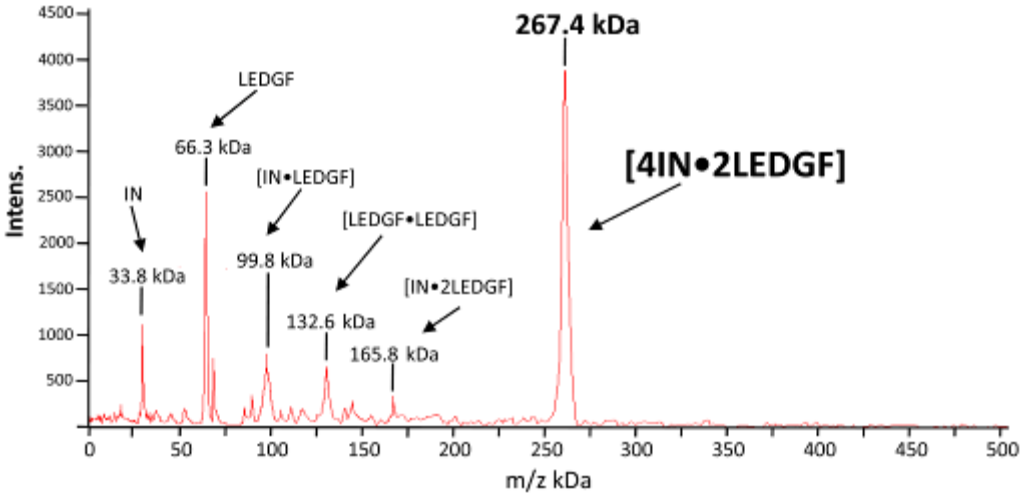
Yield: 5 mg of complex (INT 5L, LEDGF 6L)



IN/LEDGF : characterization by High Mass MALDI-TOFF



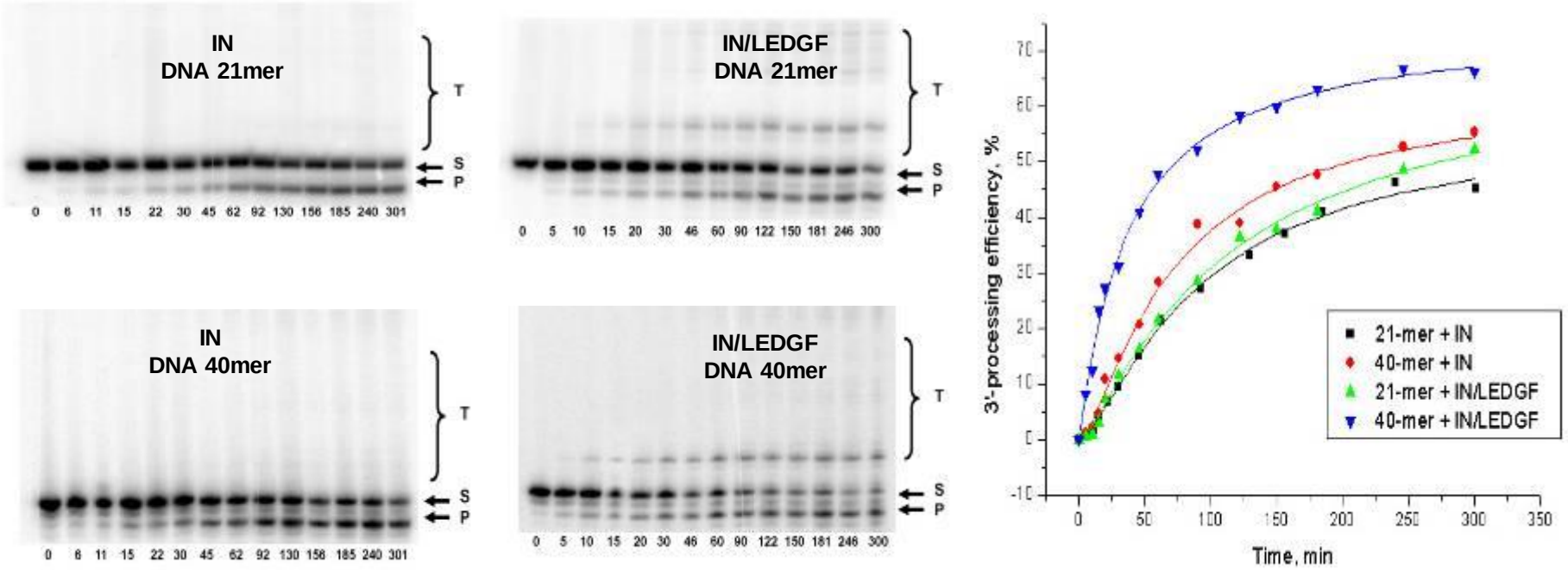
Absence of cross linking agents



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



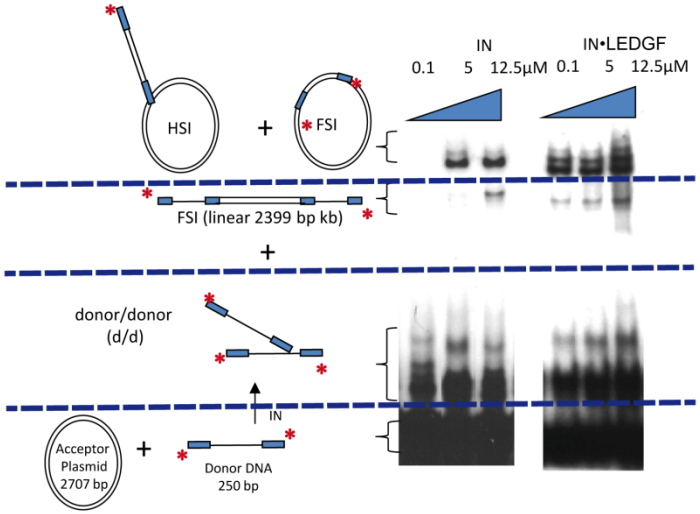
DNA: 21 or 40-mer duplex that mimic the HIV-1 U5 viral DNA end

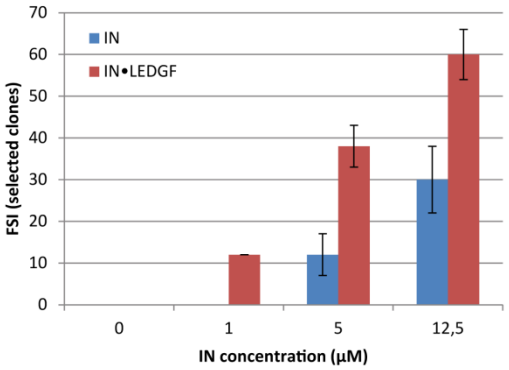
5′–**GACTACGGTTCAAGTCAGC**GTGTGGAAAATCTCTAGCAGT–3′

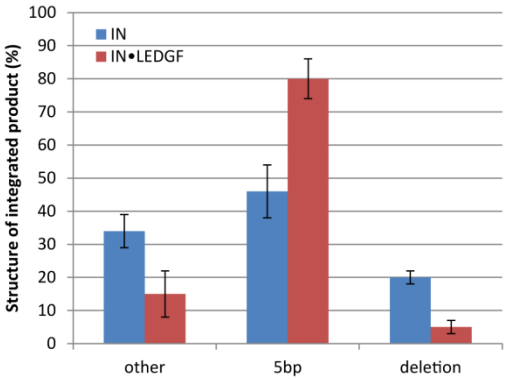
3′–**CTGATGCCAAGTTCAGTCG**CACACCTTTTAGAGATCGTCA–5′

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

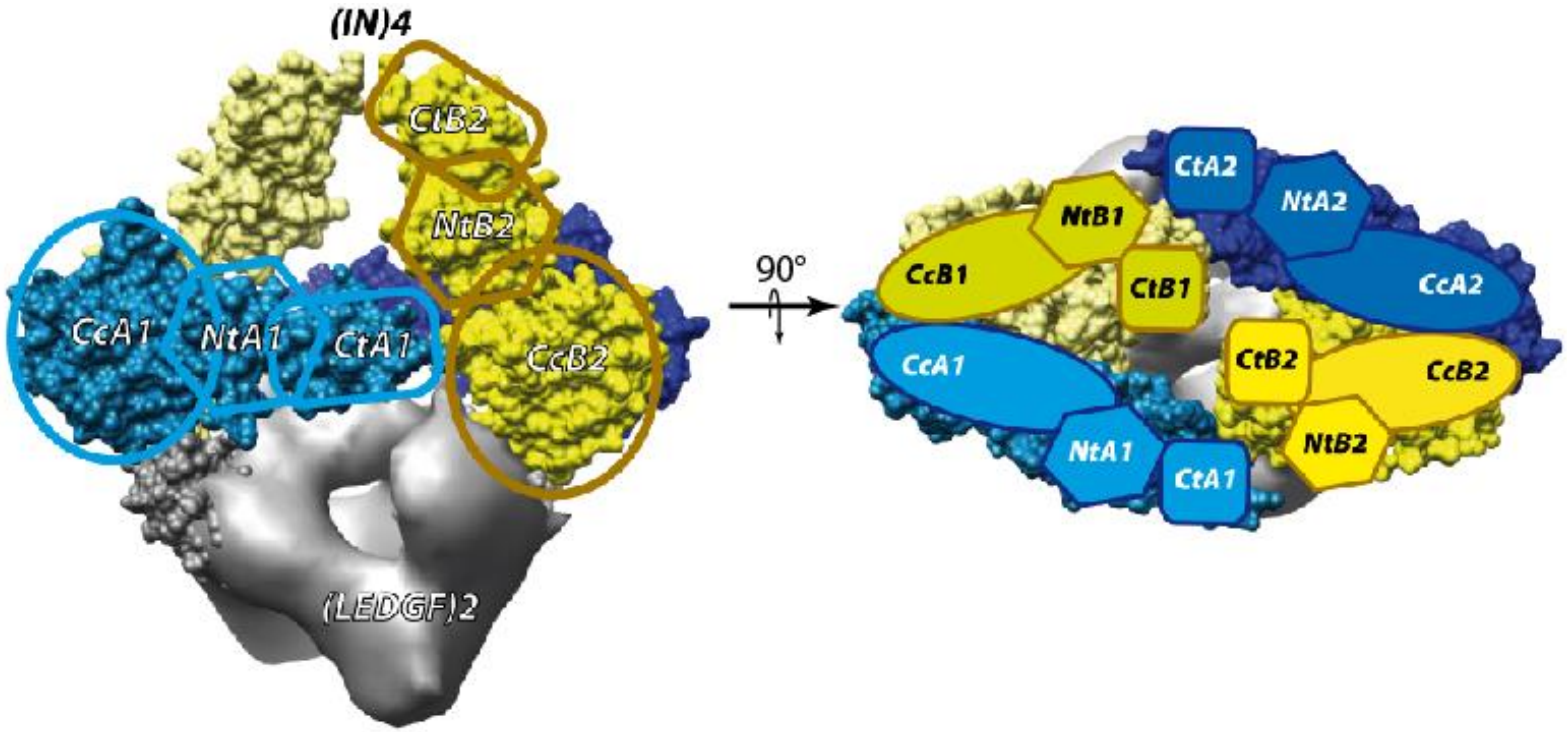






The global integration efficiency is higher for the IN/LEDGF complex than for isolated IN molecules. Specific cloning and quantification of the circular FSI products attested that the IN/LEDGF complex catalyzes more concerted integration events than isolated IN molecules. The integration reaction catalyzed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

IN/LEDGF EM Structure: Domain organization

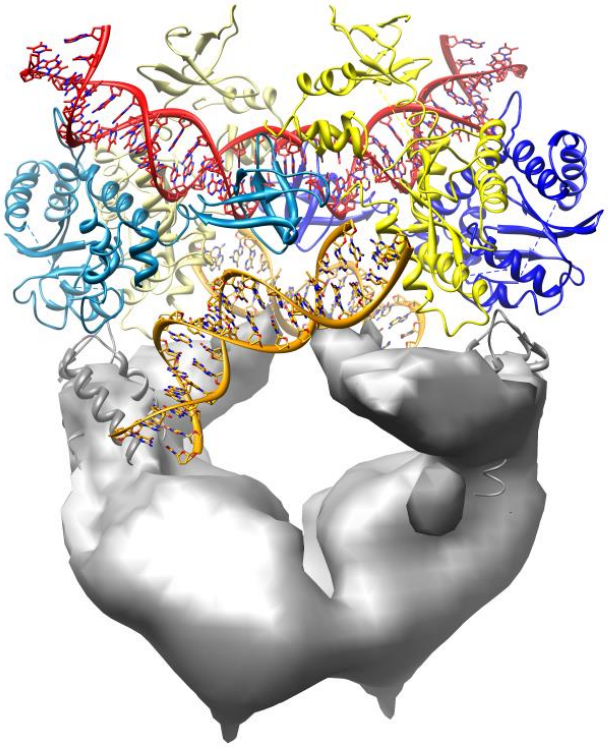


IN-LEDGF complex contains 4 IN molecules (A1, A2, B1 and B2) organized in two IN dimers

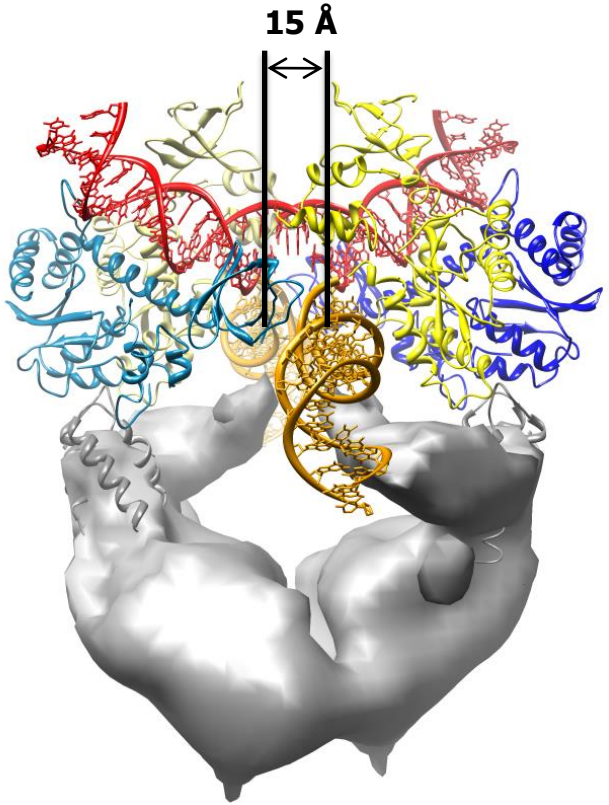
Each IN monomer within the IN dimer has a different conformation → the IN dimer is asymmetric

Each IN molecule has a distinct function within the dimer.

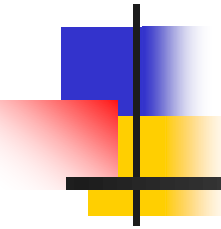
IN/LEDGF/DNA EM structure



3′ Processing

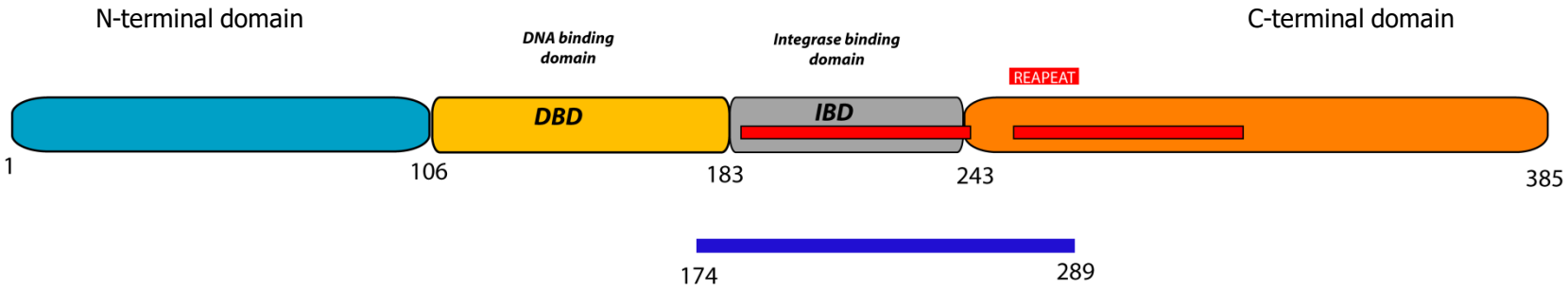


Integration



HIV-1 IN / LEDGF / INI1 complex

IN interact with INI1/SNF5

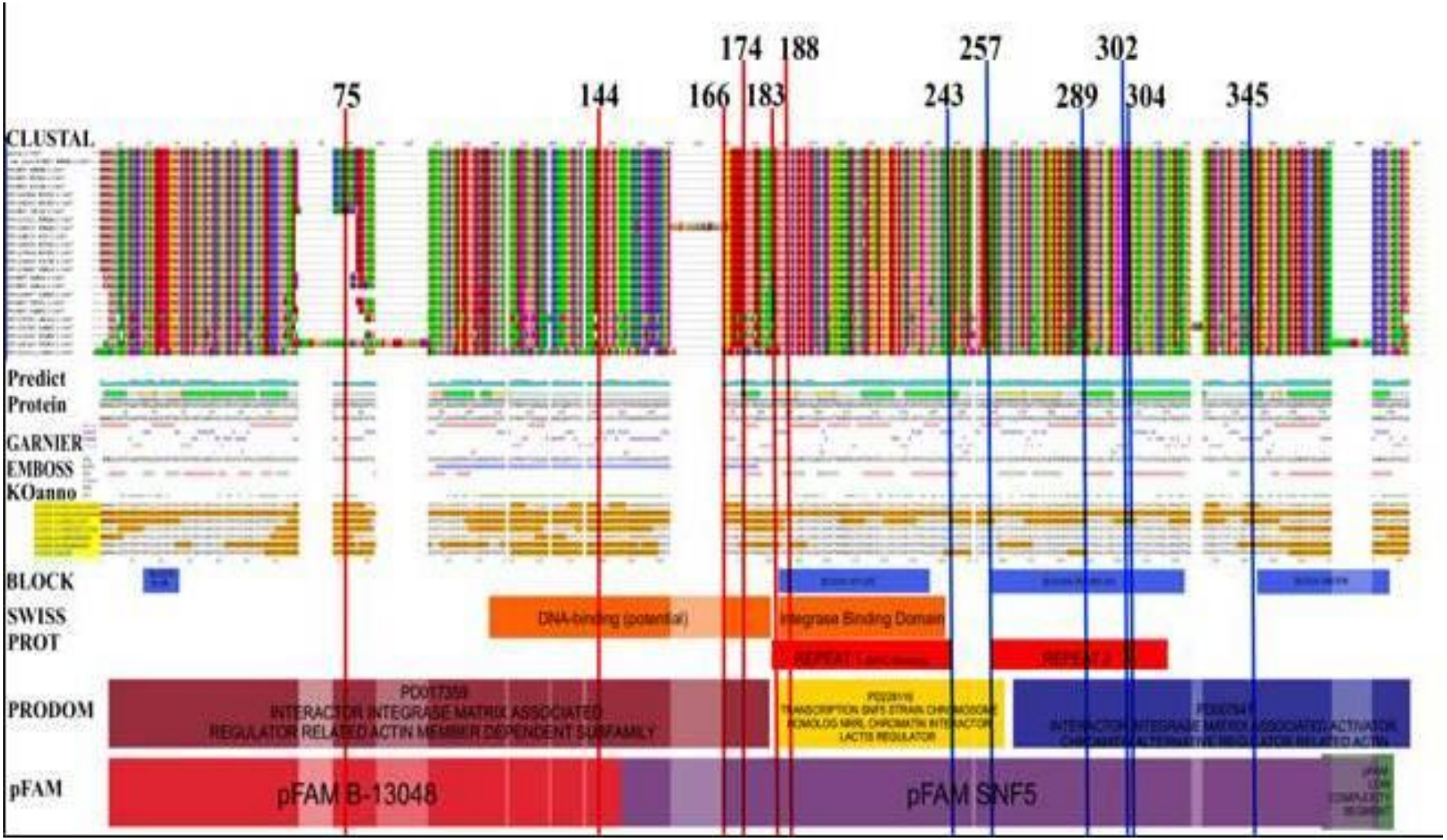


SNF5/Ini1, a subunit of the SWI/SNF chromatin remodeling complex, is the first cofactor identified to interact with IN.

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

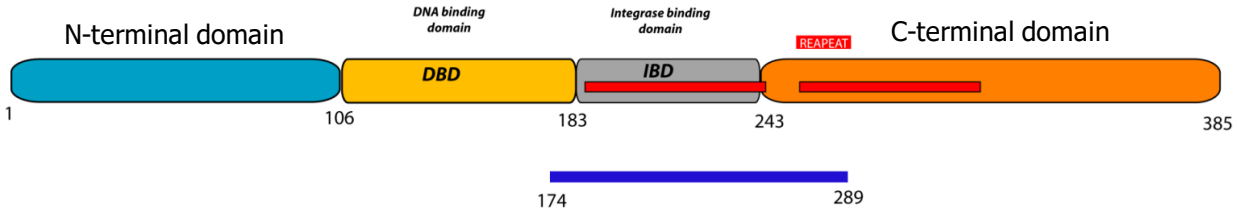
INI1/SNF5: bioinformatic analysis



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



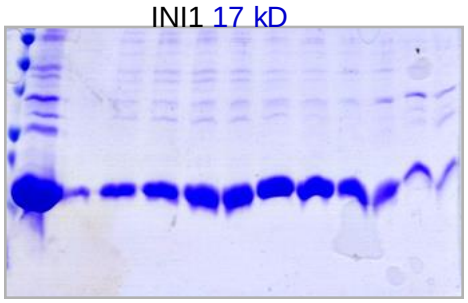
HIS-INI1(174-289) → His Affinity → Gel filtration G200

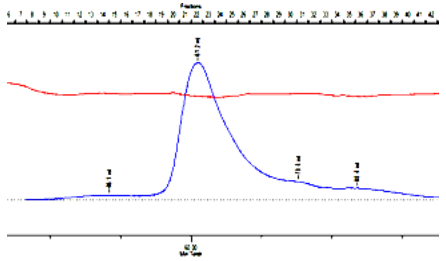
+ IN/LEDGF

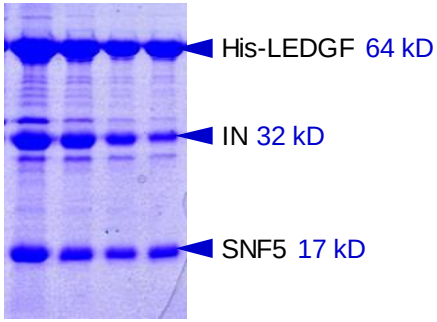
High salt detergent

Gel filtration G200

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



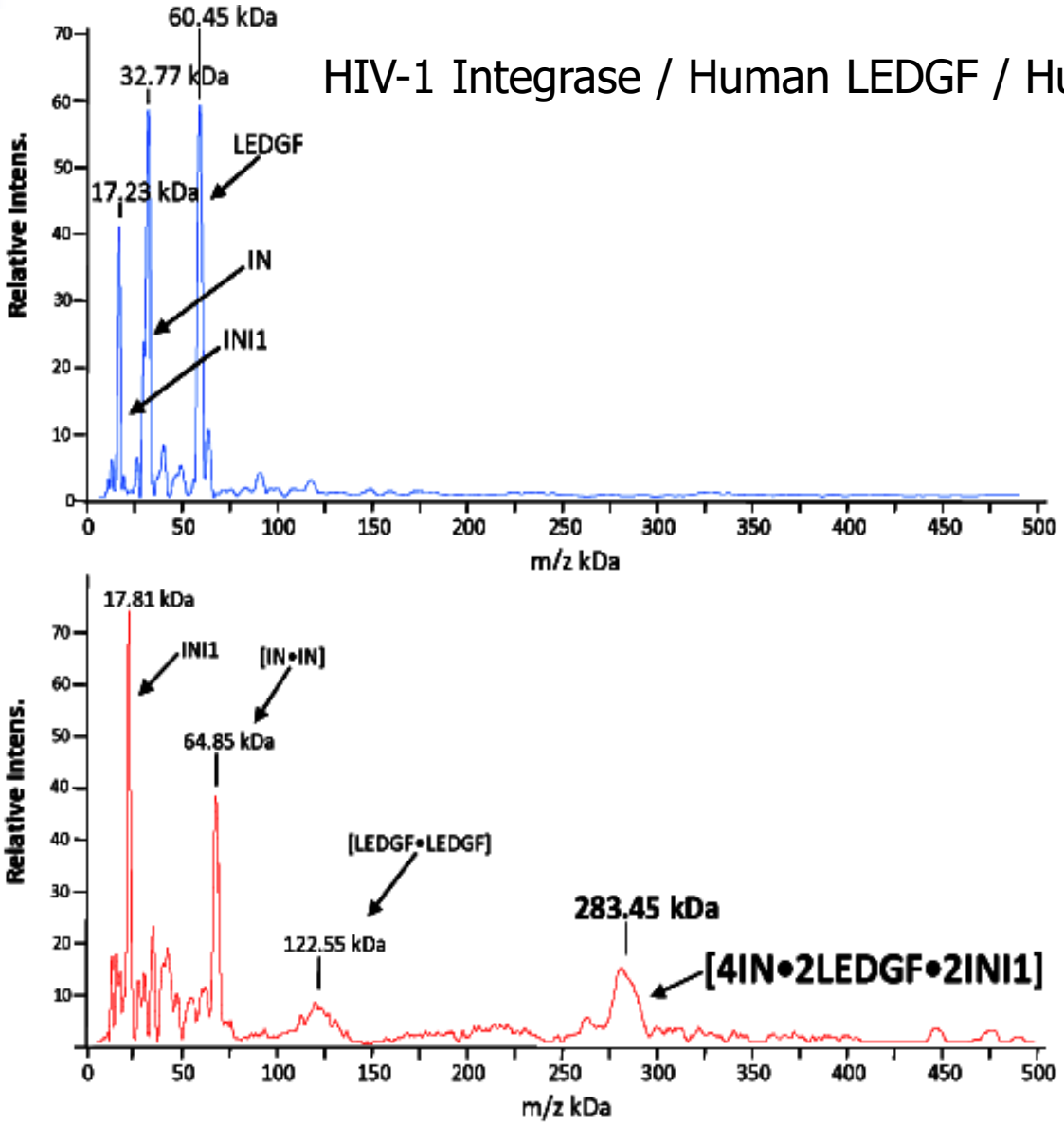




Yield: 2.5 mg of complex (INT 0.5L, LEDGF 0.5L, SNF5 1.0L)

Protein complexes analysis : High Mass MALDI-ToF

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



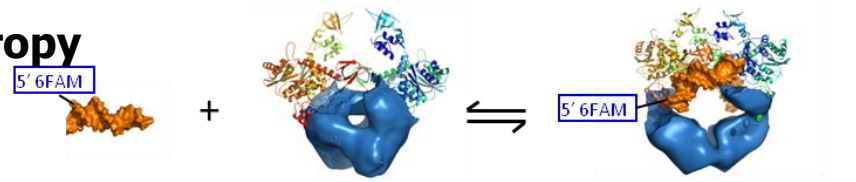
IN/LEDGF and IN/LEDGF/INI1: Functional characterization

Viral DNA Binding assay by fluorescence anisotropy

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

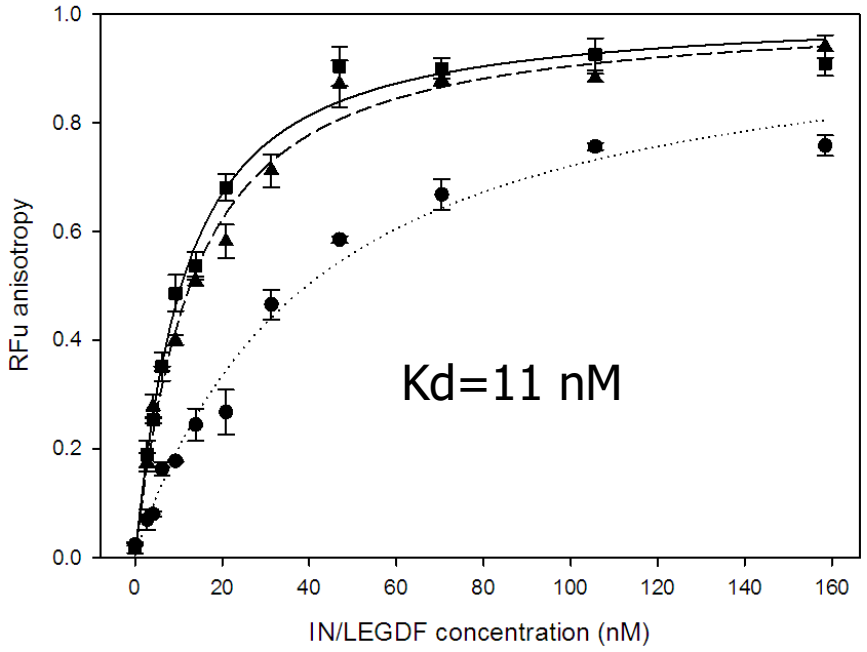
5′-GACTACGGTTCAAGTCAGCGTGTGGAAAATCTCTAGCAGT-3′

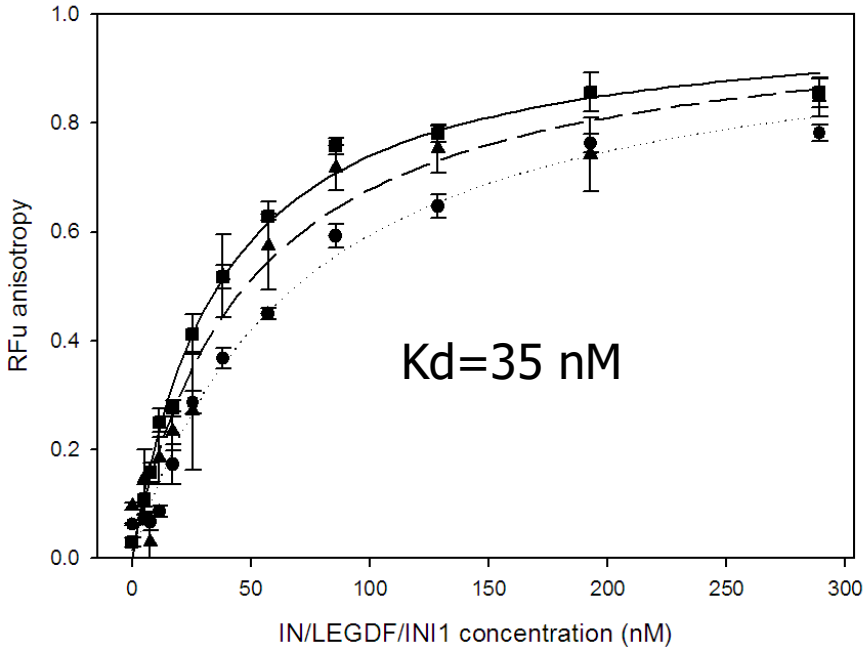
3′-CTGATGCCAAGTTCAGTCGCACACCTTTTAGAGATCGTCA-5′



Fast rotation: low anisotropy

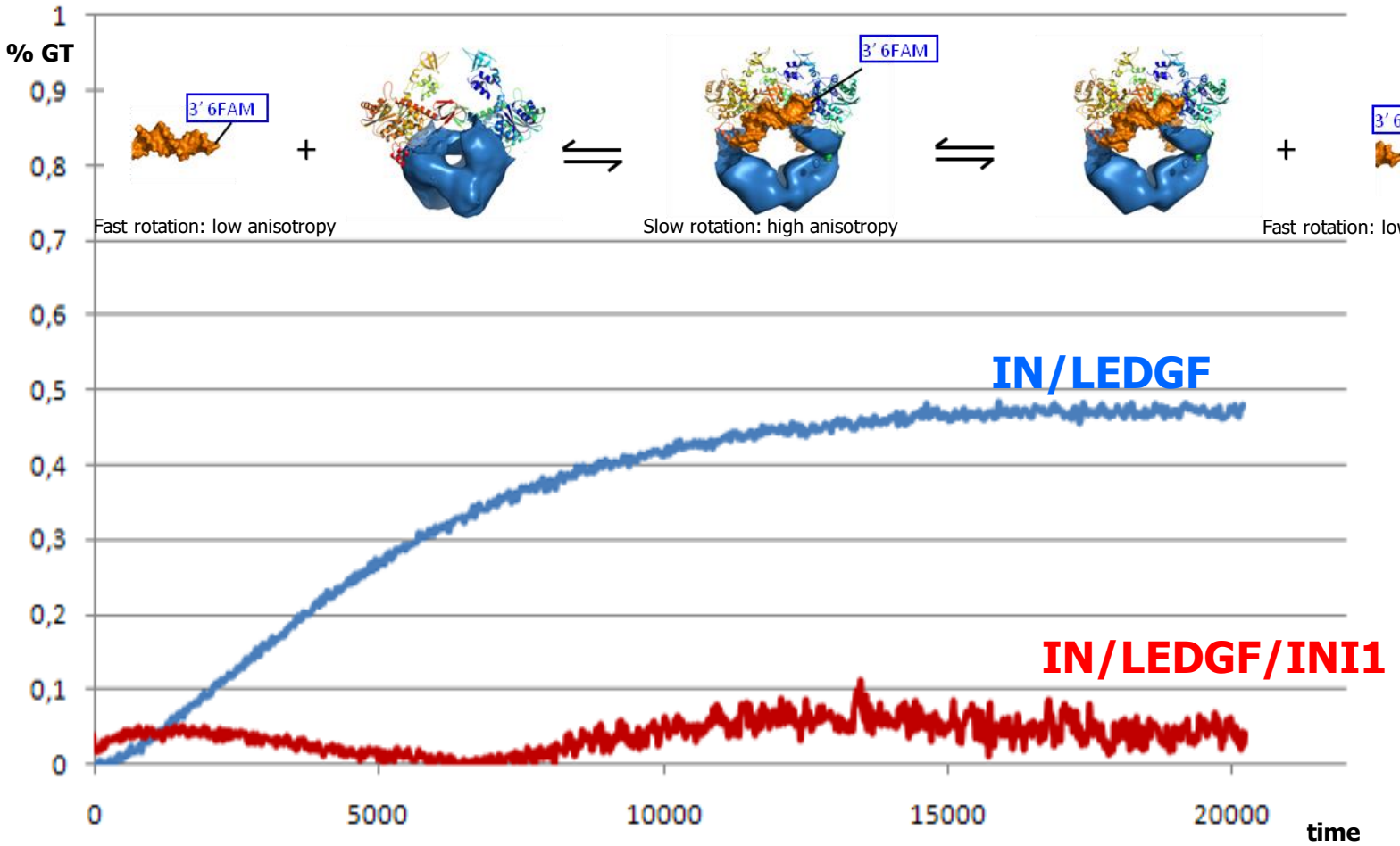
Slow rotation: high anisotropy





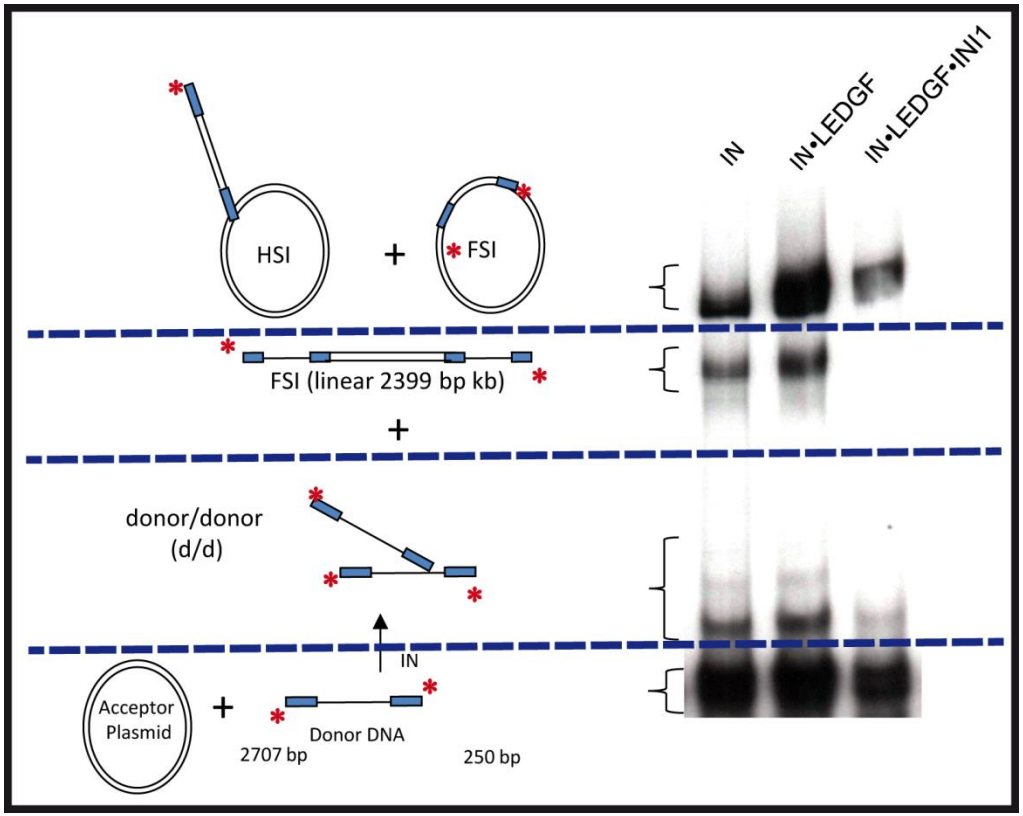
IN/LEDGF and IN/LEDGF/INI1: Functional characterization

The 3′ Processing assay by fluorescence anisotropy



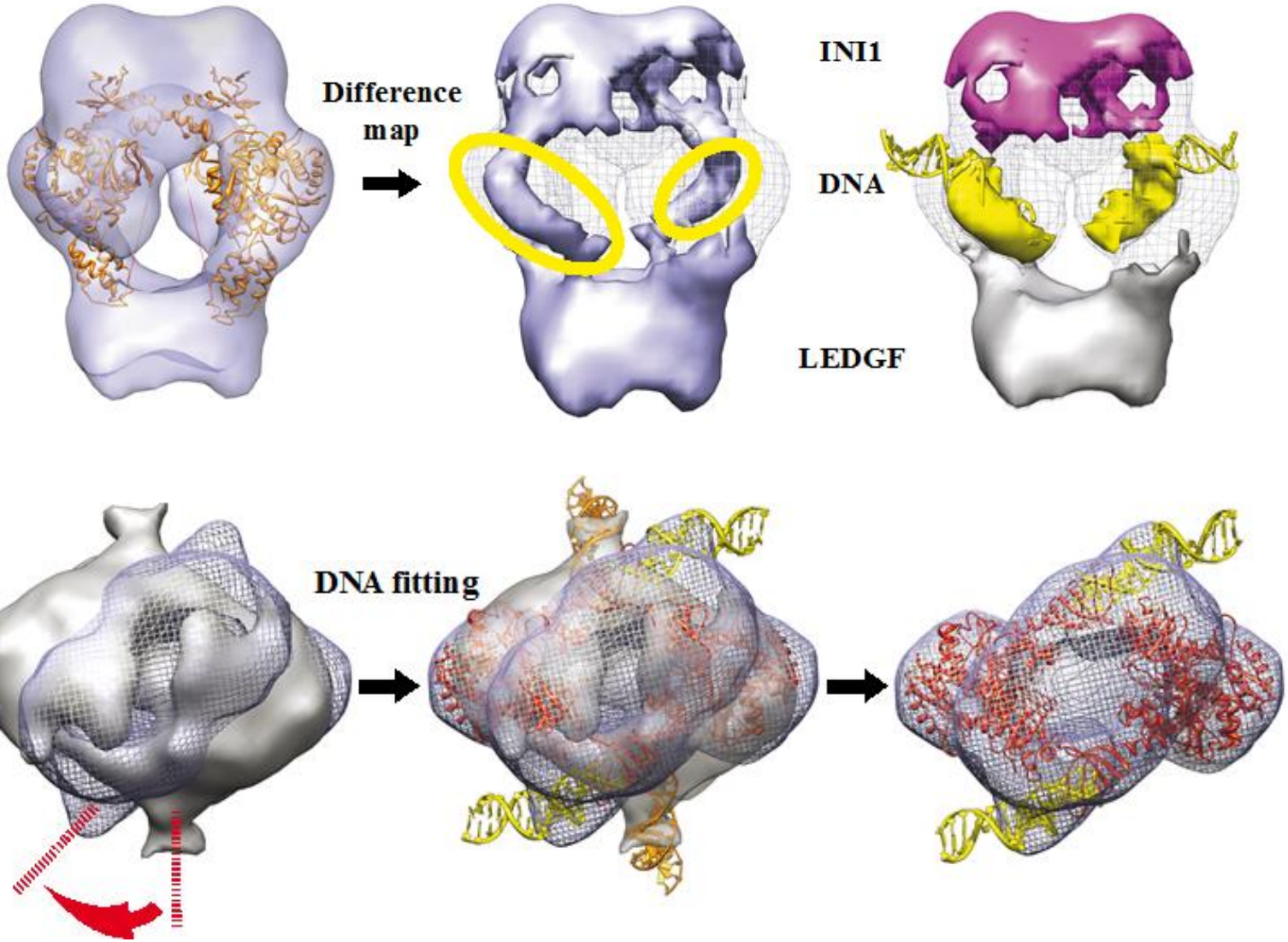
INI1 inhibits the *3′ processing* activity of IN/LEDGF

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

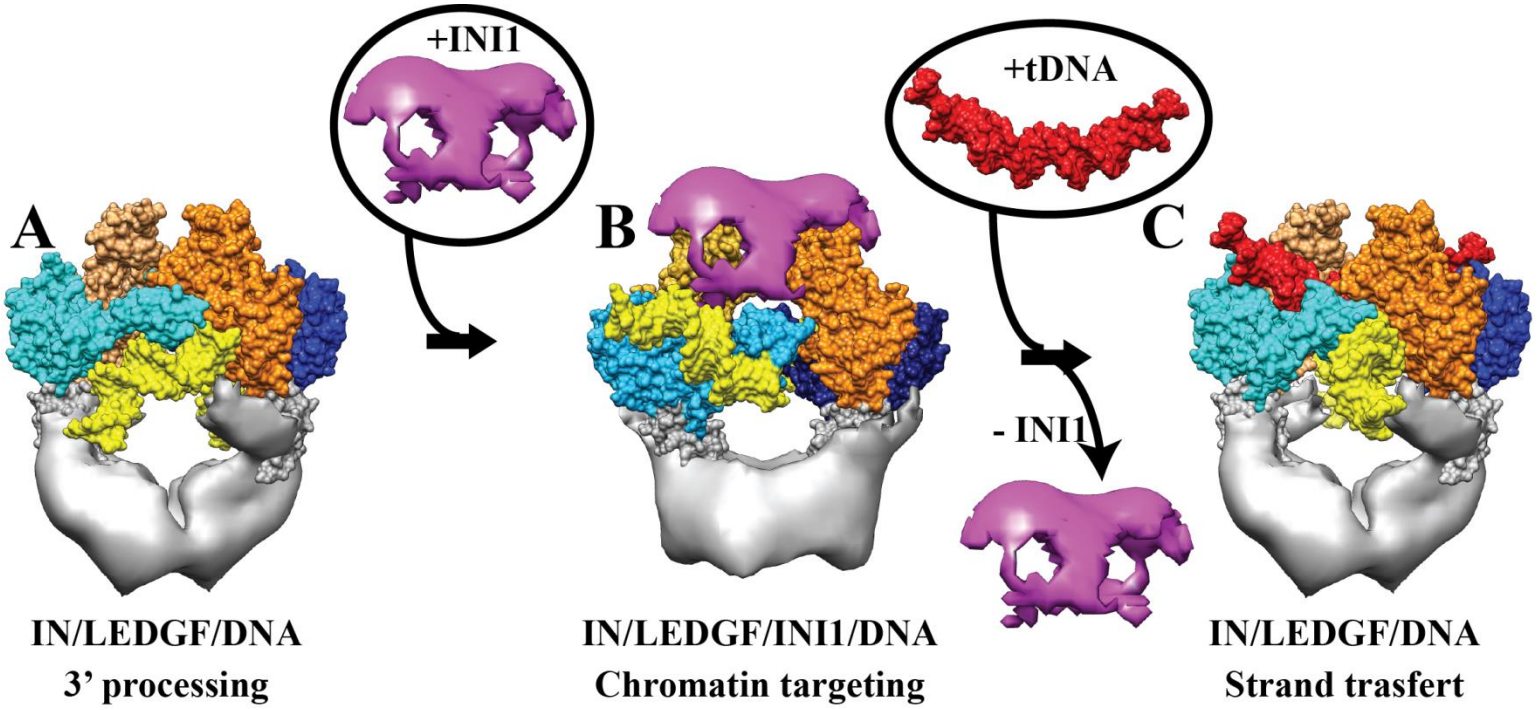


In the presence of INI1, integration occurs with reduced kinetics compared to IN alone or to the IN/LEDGF complex with strongly reduced by-products formation

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



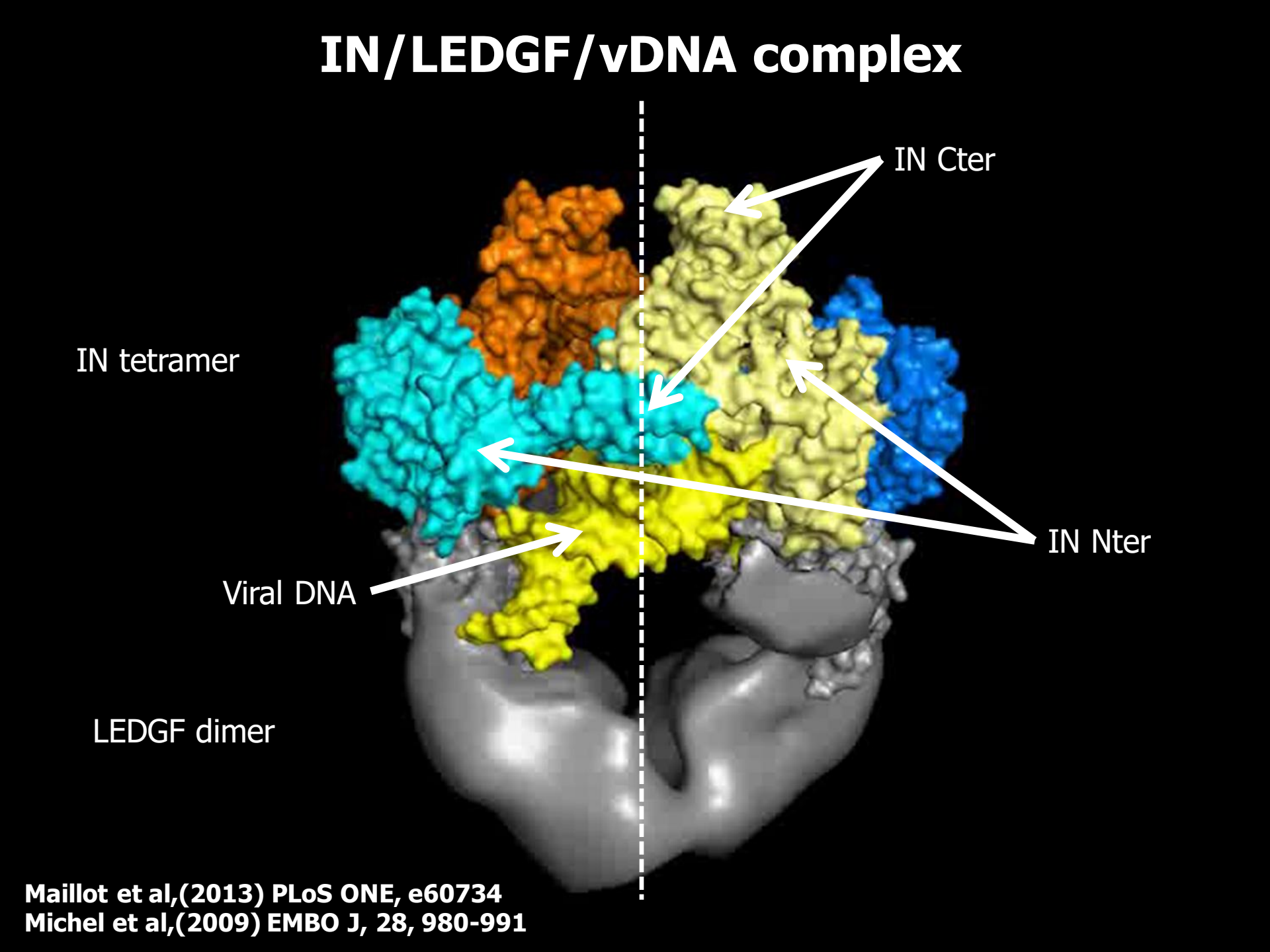
Function of INI1 in HIV-1 infection



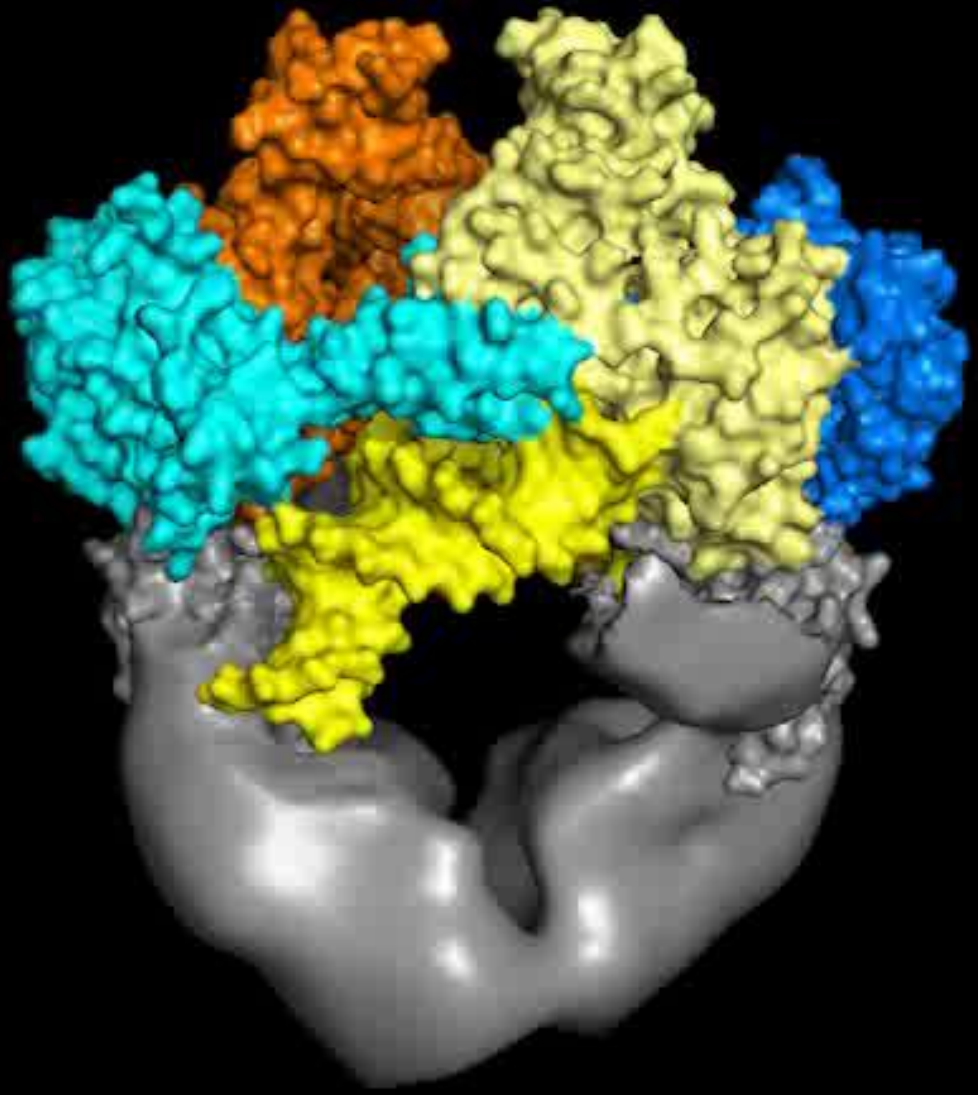
Benoit Maillot, Nicolas Lévy, Sylvia Eiler, Corinne Crucifix, et al., (2013), Structural and functional role of INI1 and LEDGF in the HIV-1 preintegration complex, PlosOne, In Press

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

IN/LEDGF/vDNA complex

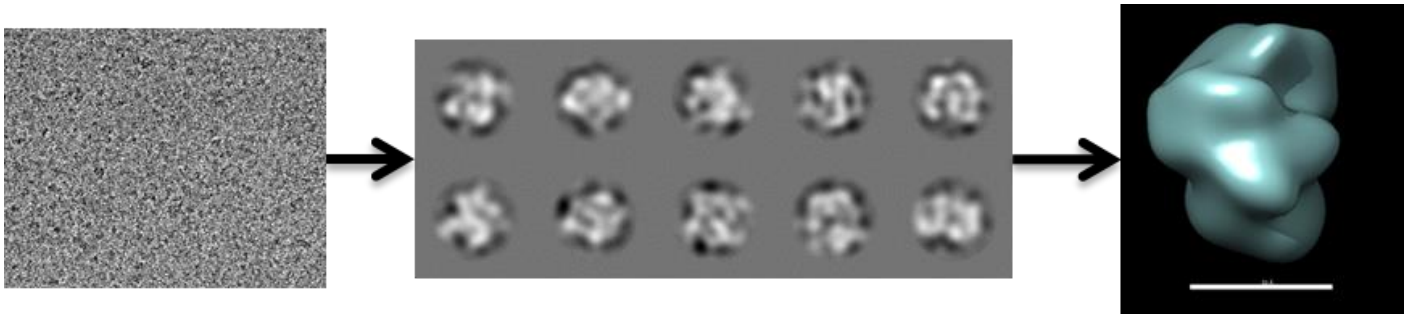


Maillot et al,(2013) PLoS ONE, e60734
Michel et al,(2009) EMBO J, 28, 980-991



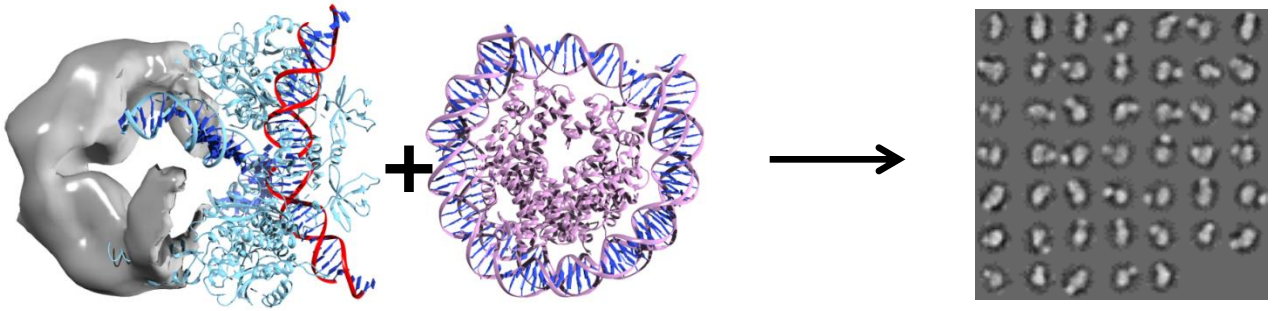
Other PIC complexes….. In progress

IN nuclear import/export: IN interacts with TNPO3, CA, VBP1



Preliminary CryoEM data on IN/TNPO3/VBP1

Integration: IN interacts with the nucleosome



IN/LEDGF/DNA complex Nucleosome Preliminary Negative stained EM

Structure and function of HIV Integrase domains

Histone H4K20Me1 interacts with HIV-1 IN C-terminal domain

H4K20Me1 interacts with HIV-1 IN

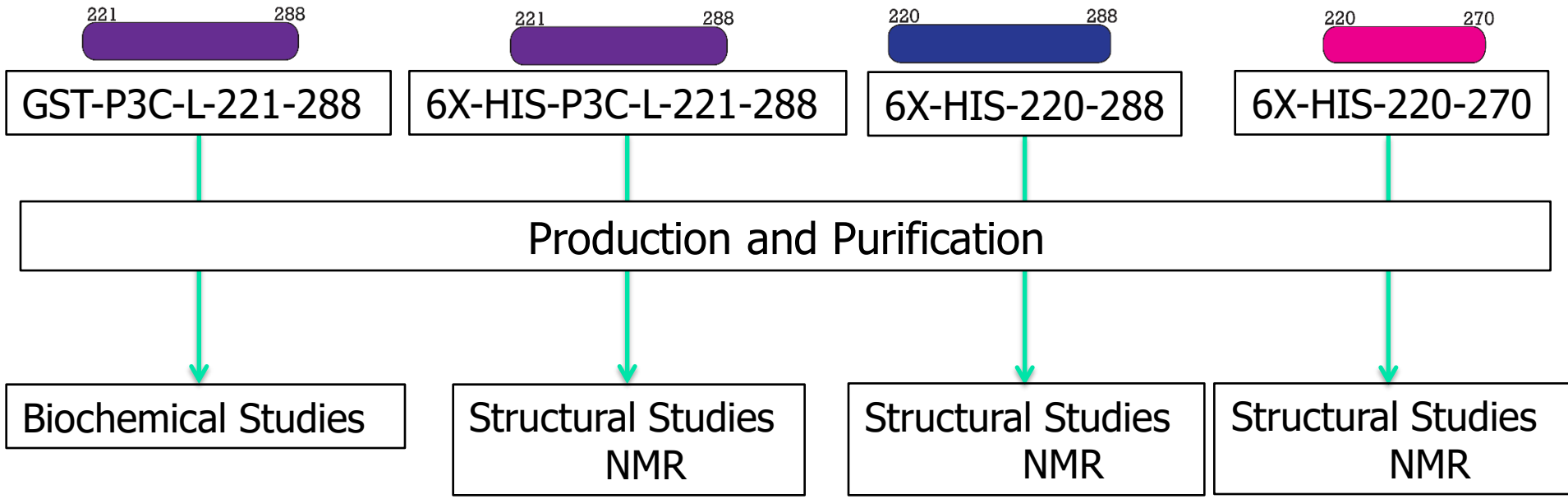
Histone peptide array

Evidence that there is a specific and functional interaction between IN-CTD and the mono-methylated N-terminal tail of H4K20

- **Structural basis of the interaction between IN-CTD and H4K20Me1**

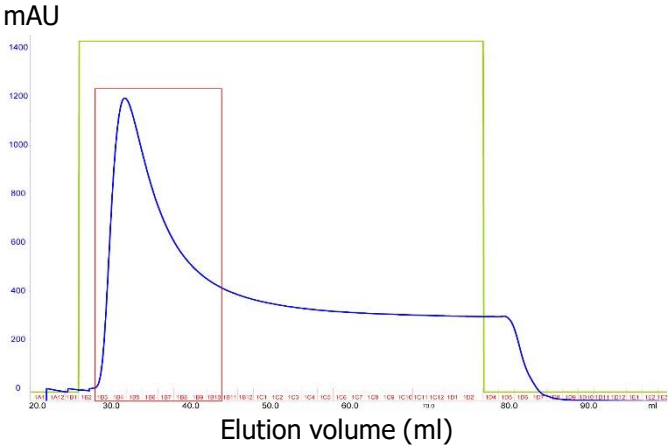
Matysiak et al., submitted

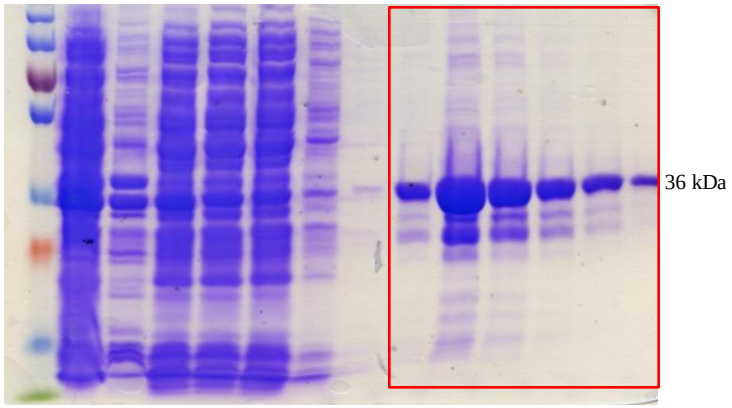
Cloning of IN-CTD fragments



GST-CTD (221-288) HIV-1 Integrase purification

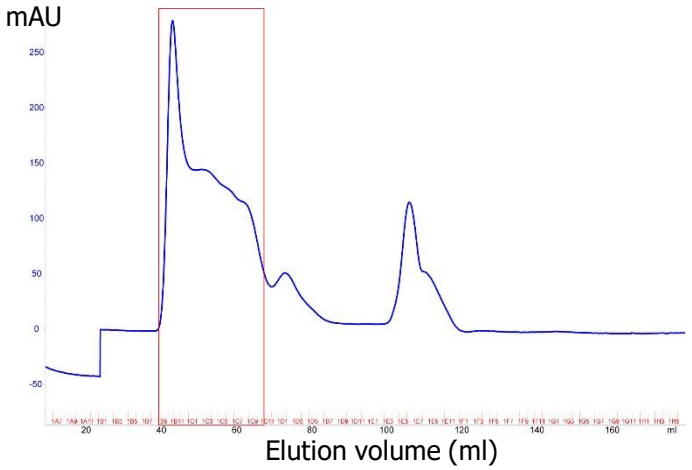
Affinity Chromatography

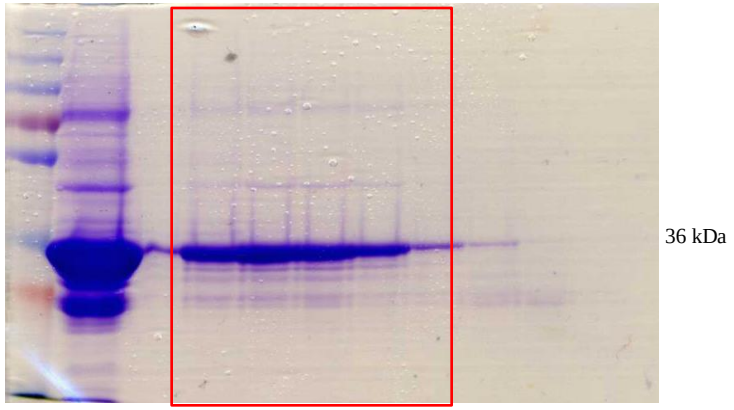




Protein was solubilized and purified in 25mM HEPES pH 8, 2mM $MgCl_2$, 2mM βME, 150mM NaCl, 100mM Arginine

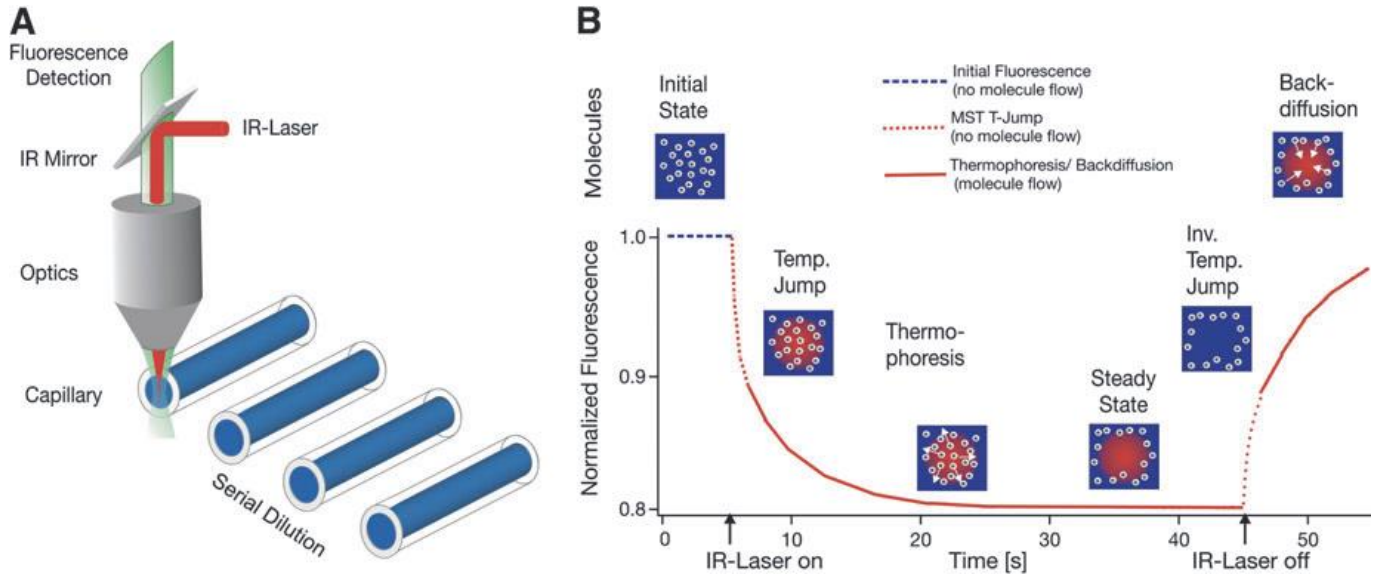
Size-Exclusion Chromatography





Pooled fractions were purified using Superdex S200 in 25mM HEPES pH 8, 2mM $MgCl_2$, 2mM βME, 150mM NaCl.

Microscale thermophoresis for Kd measurement



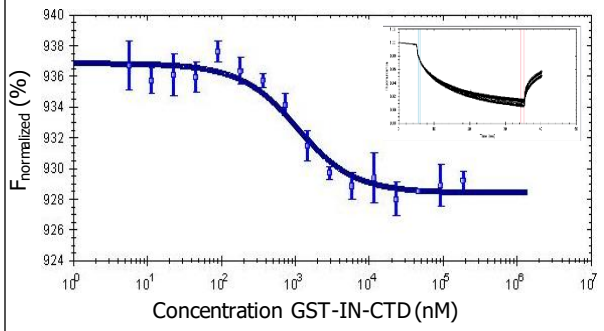
(A) MST is measured in a capillary with a total volume of 4 mL. The fluorescence within the capillary is excited and measured through the same optical element. An IR-Laser is used to locally heat the sample volume that is observed by fluorescence. T-Jump and thermophoresis are directly observed as a change in fluorescence at different time scales.

(B) A typical MST signal for a given capillary. Initially, the molecules are homogeneously distributed and a constant "initial fluorescence" is measured. As soon as the IR-Laser is turned on, a fast T-Jump is observed, followed by thermophoretic molecule motion. The fluorescence decrease is measured for about 30 s. When the IR-Laser is turned off, an inverse T-Jump is observed, followed by the "backdiffusion" of molecules, which is purely driven by mass diffusion, allowing to deduce information on the molecule size.

Microscale Thermophoresis confirm a specific interaction between H4K20Me1 and GST-CTD HIV-1 Integrase

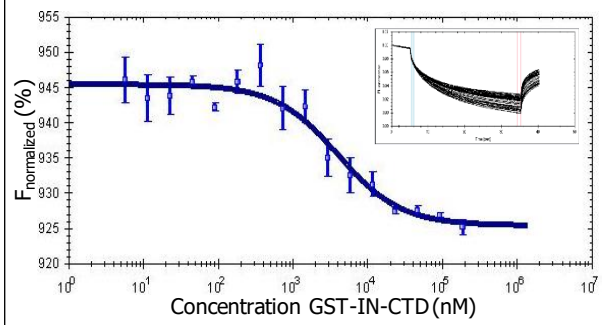
A

H4K20Me1, K_d = 0.8µM± 0.13



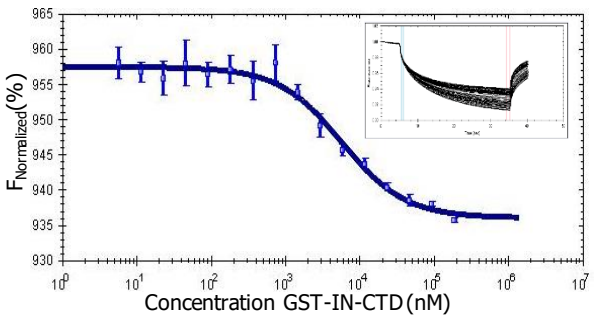
B

H4K20Me2, K_d= 3.75µM± 0.6



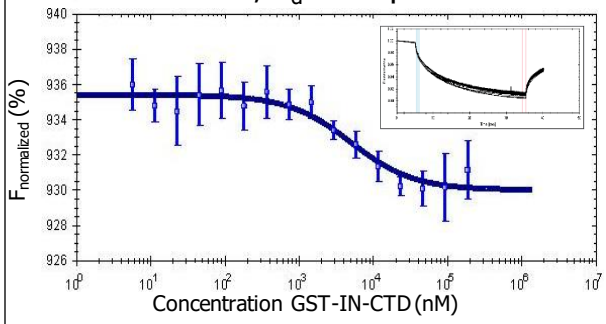
C

H4K20Me3, K_d = 5.2µM± 0.5



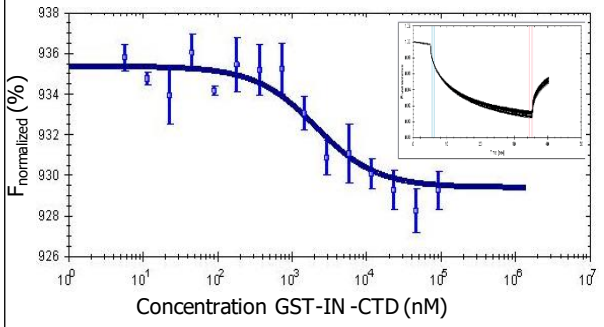
D

H4K20Me0, K_d = 4.7µM± 1.0



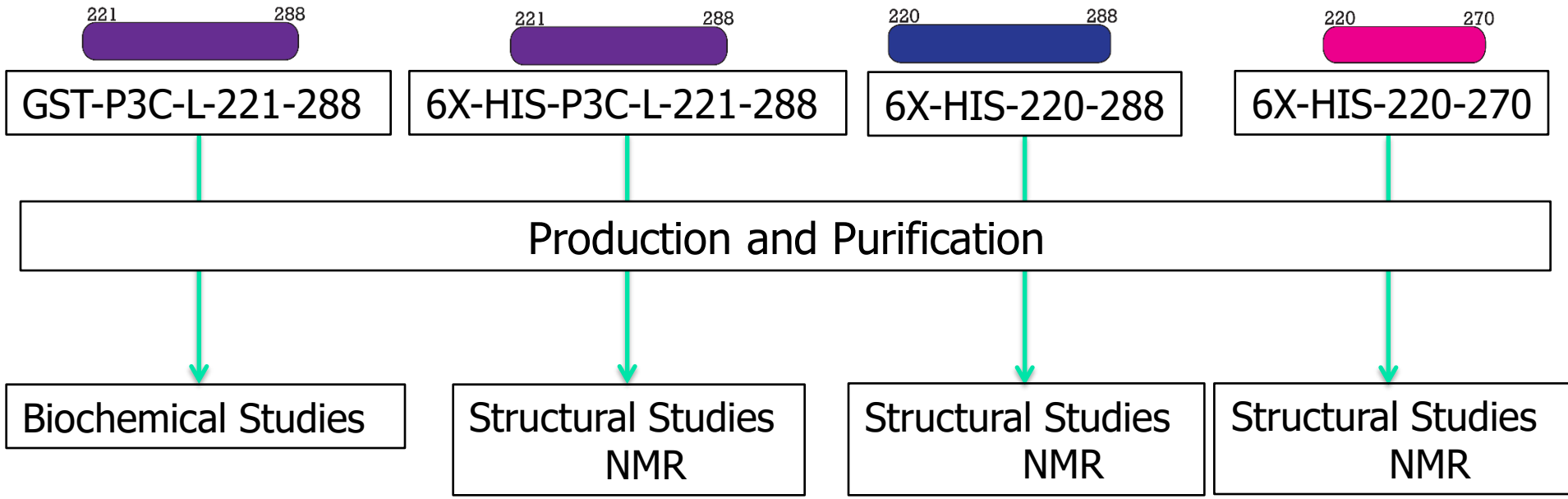
E

H4K20Me1+H4K20Me0, K_d= 1.8µM± 0.4



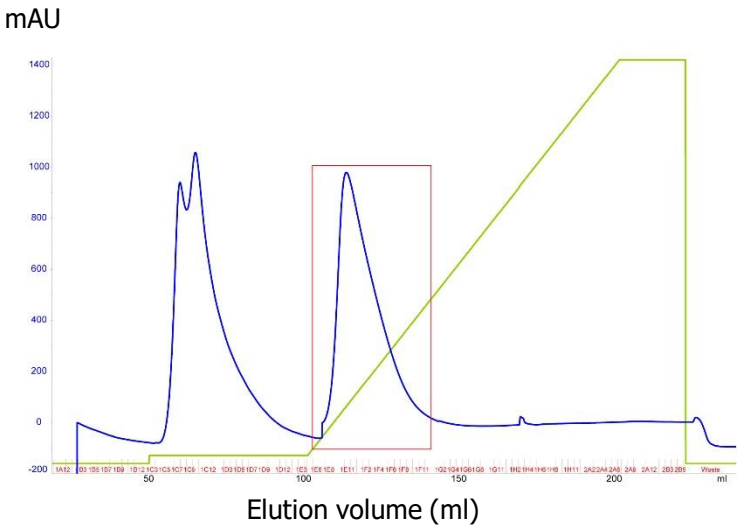
MST experiments were carried out with Fluo-KGG-RHRK(me_x)VLR

Cloning of IN-CTD fragments

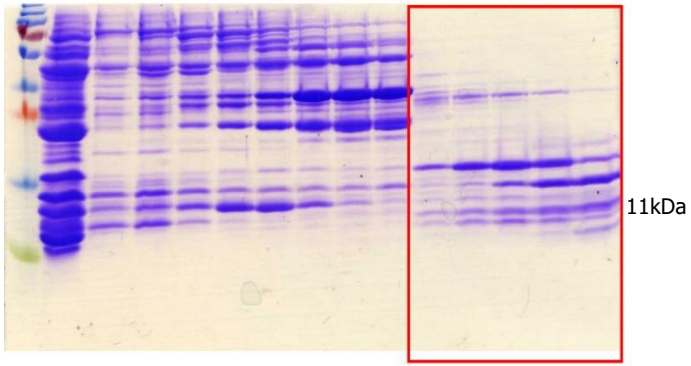


NMR: HIS-P3C-IN 221-288

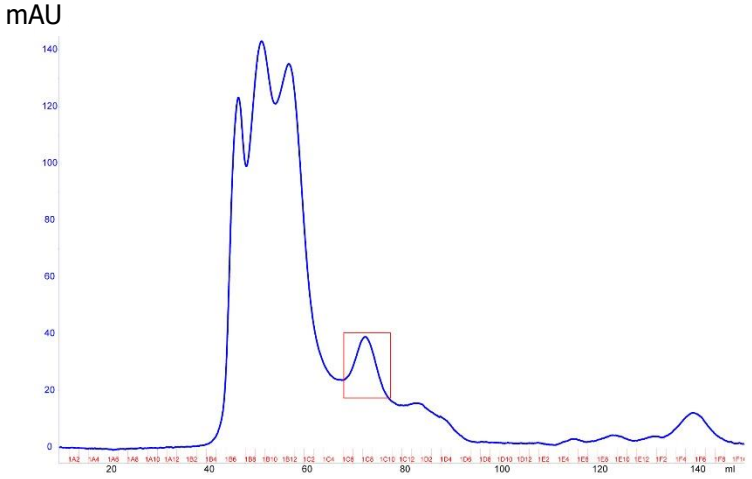
Affinity Chromatography



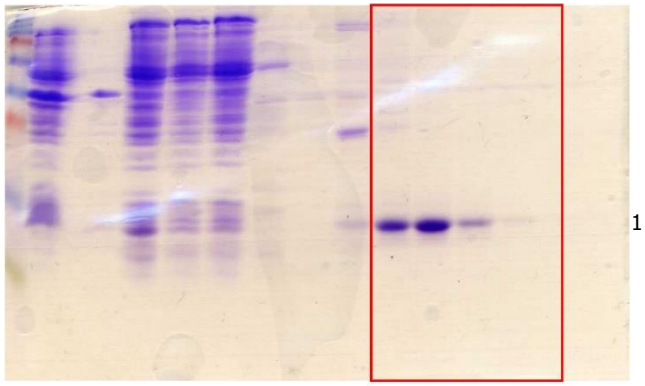
Elution volume (ml)



Size-Exclusion Chromatography



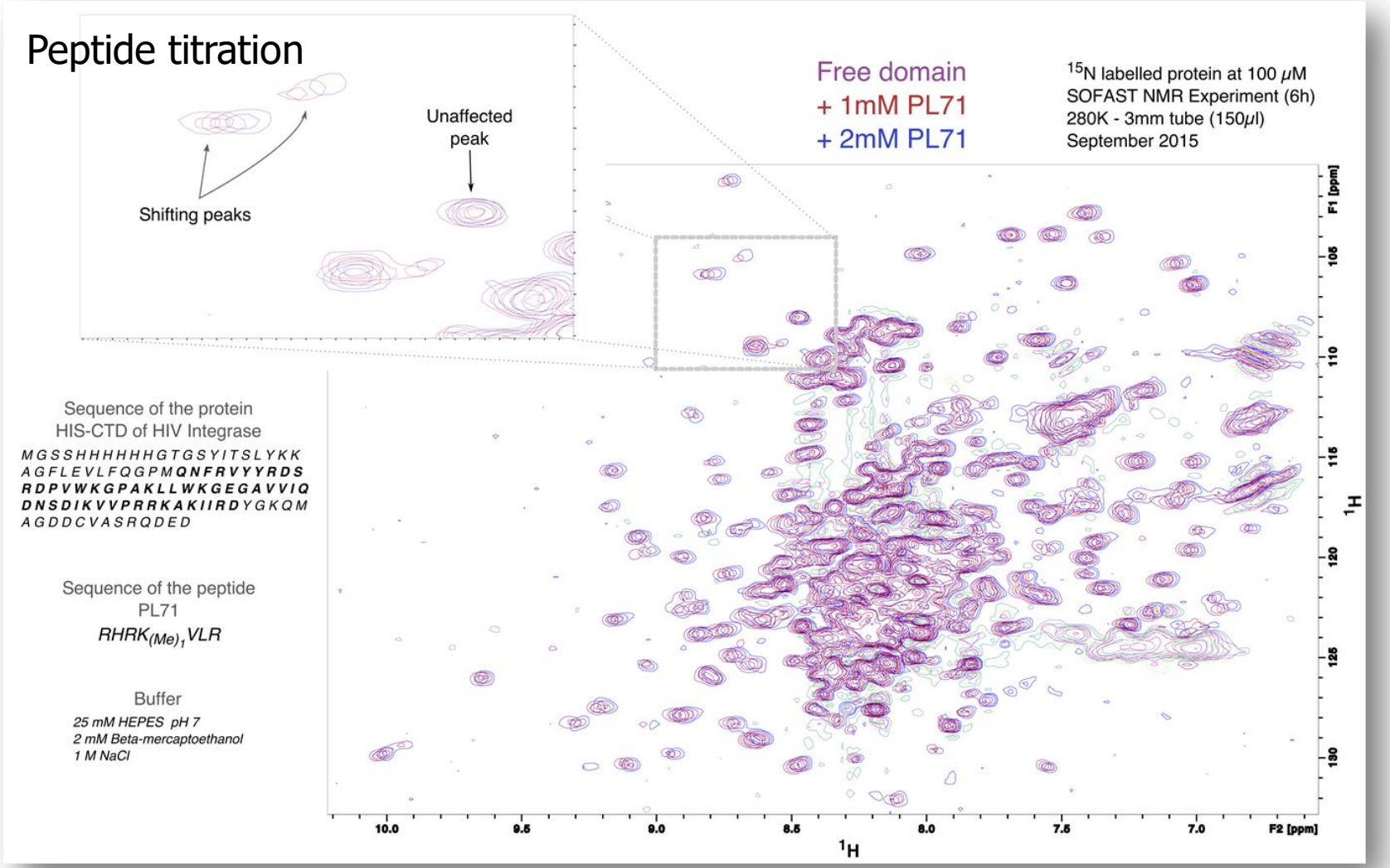
Elution volume (ml)



Protein was solubilized and purified in 25mM HEPES pH 7, 2mM βME, 1M NaCl

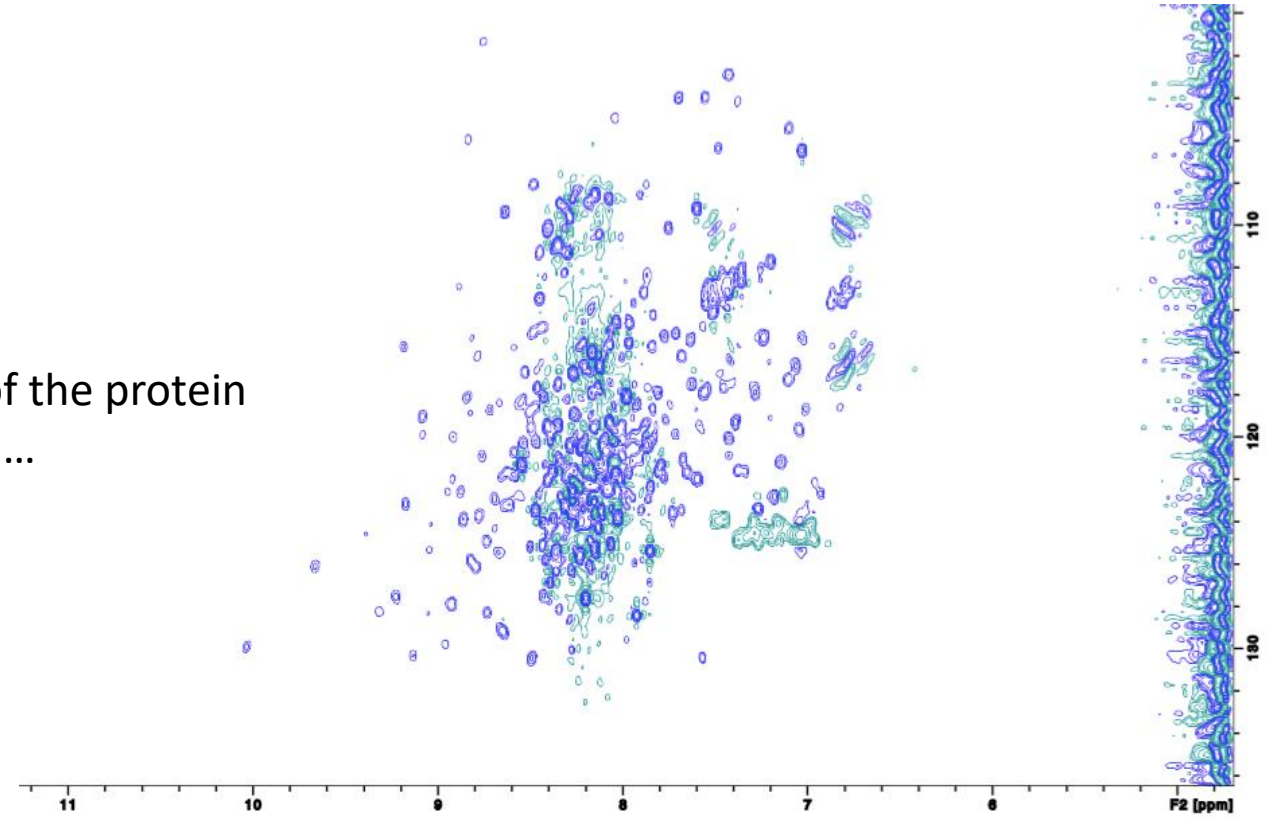
Pooled fractions were purified using Superdex S75 in 25mM HEPES pH 7,2mM βME, 1M NaCl

HIS-P3C-IN 221-288: HSQC spectra



HIS-P3C-IN 221-288 HSQC spectra

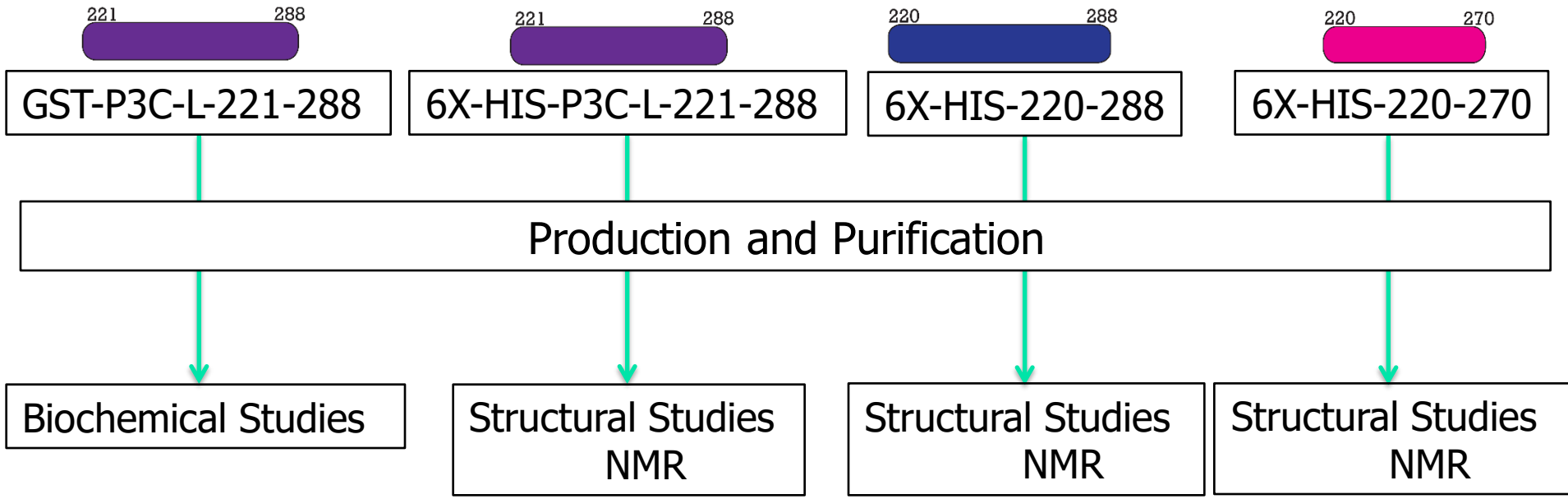
A section of the protein is unfolded…



HIS-P3C-IN 221-288：

M G S S H H H H H H G T G S Y I T S L Y K K
A G F L E V L F Q G P M ^{221}Q N F R V Y Y R
D S R D P V **W** K G P A K L L **W** K G E G A V
V I Q D N S D I K V V P R R K A K I I R D$_{270}$
Y G K Q M A G D D C V A S R Q D E D$_{288}$

Cloning of IN-CTD fragments

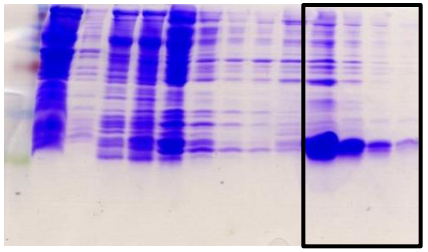


HIS-P3C-IN 220-288, 270

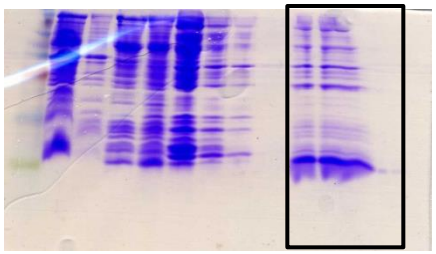
Culture in N15 labeled minimal medium. Induced with 0.5mM IPTG for 4 hours

HIS-TRAP 25mM HEPES pH7, 2mM bME, 1M NaCl as lysis buffer, and 25mM HEPES pH7, 2mM bME, 1M NaCl, 500mM Imidazole

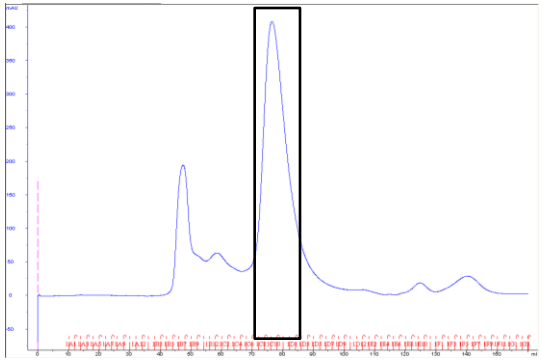
HIS 220-288 – 8.98kD

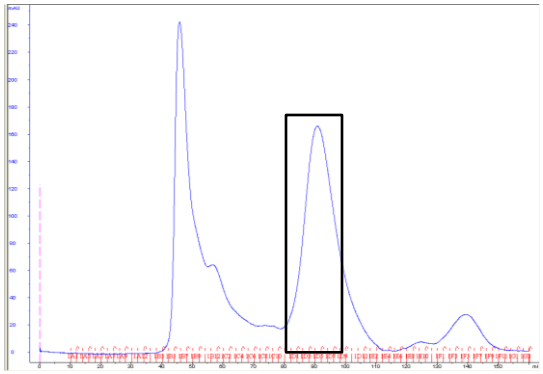


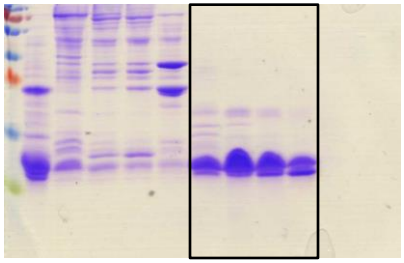
HIS 220-270 - 7.6kd

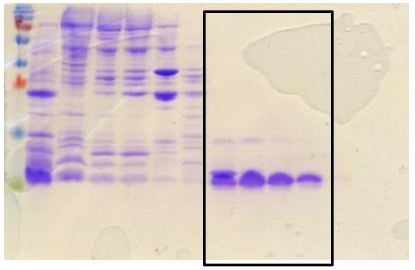


Pool fractions and run GF using S75 16/60 into 25mM HEPES pH7, 2mM bME, 1M NaCl







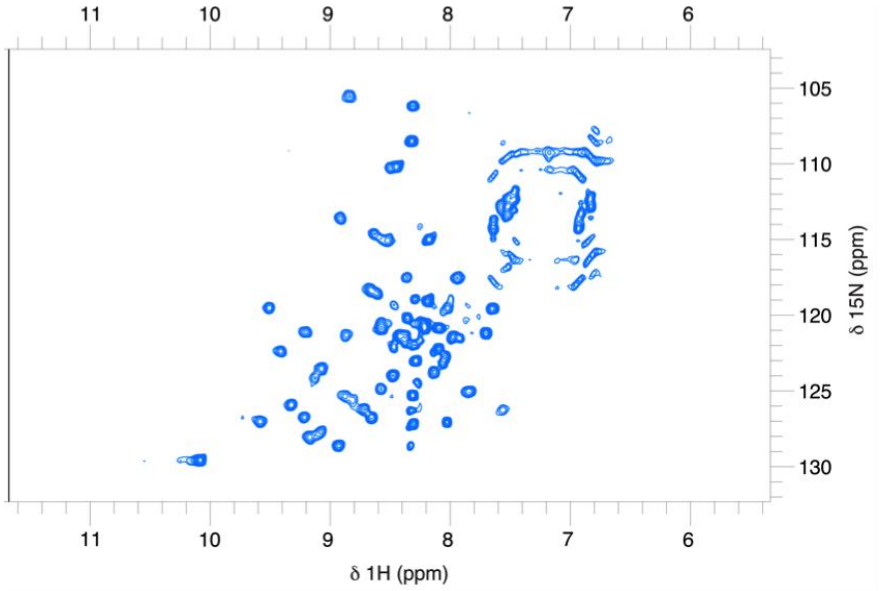


HIS-P3C-IN 220-270, 288: HSQC spectra

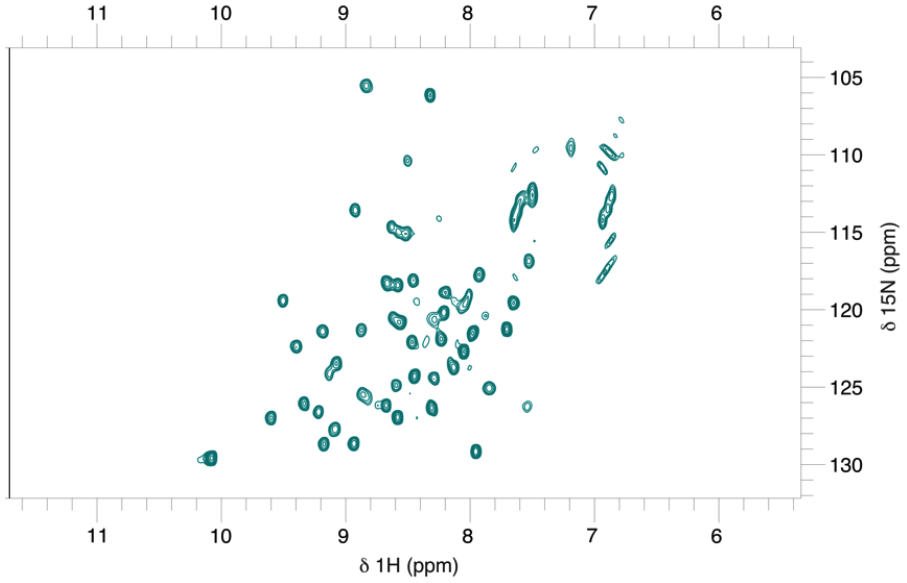
M G H H H H H H ^{220}I Q N F R V Y Y R D S R D P V W K G P A K L L W K G E G A V V I Q D N S D I K V V P R R K A K I I R D_{270} Y G K Q M A G D D C V A S R Q D E D_{288}

220-288, pH 7

220-270, pH 7



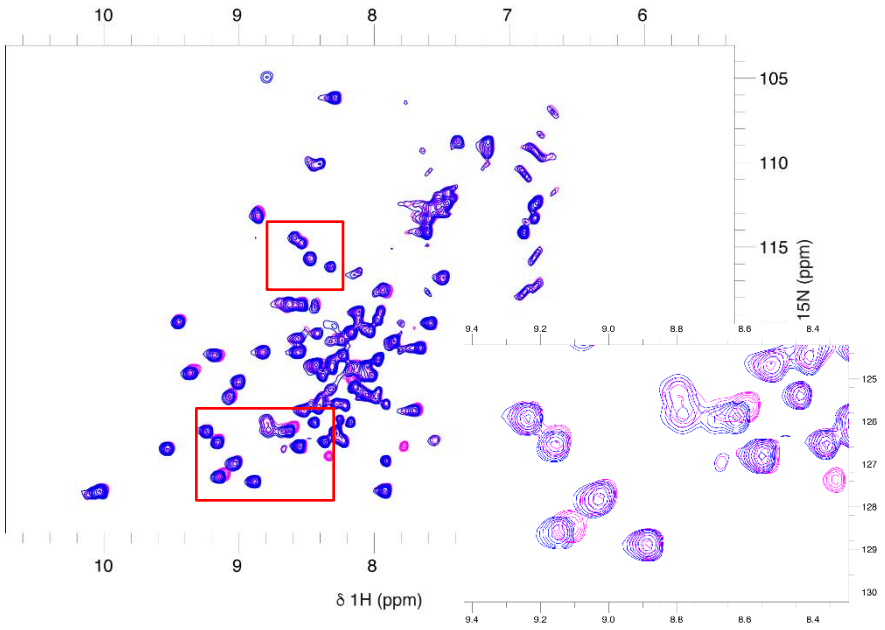
More disordered



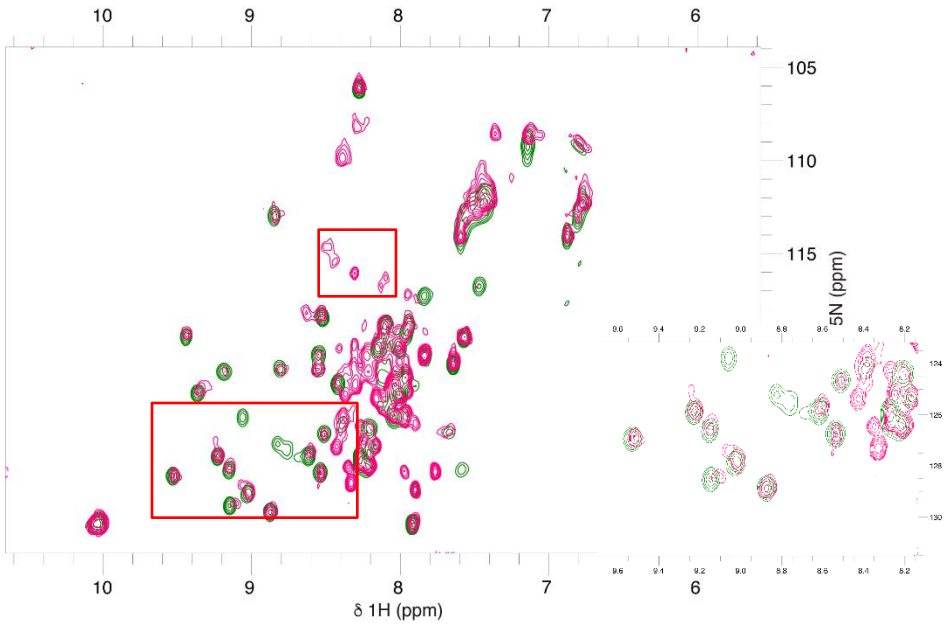
Better ordered

HIS-P3C-IN 220-270: HSQC spectra, pH 7 and 8, +/- peptide

220-270 + 2mM Peptide, pH 7



220-270 + 2mM peptide, pH 8



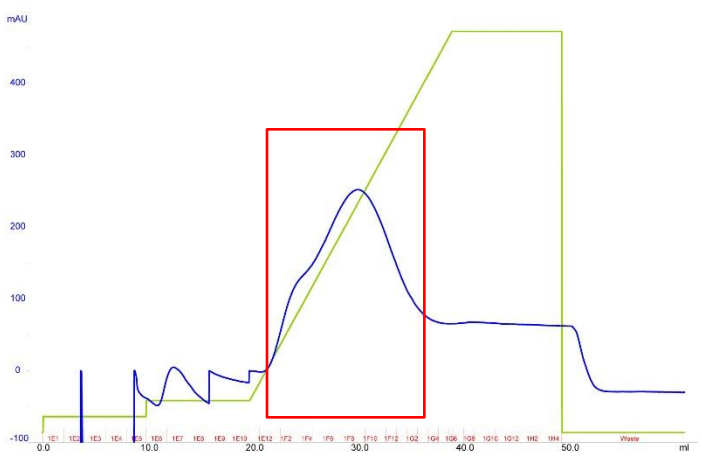
Protein only pH 7 (blue) plus 2mM peptide (pink)

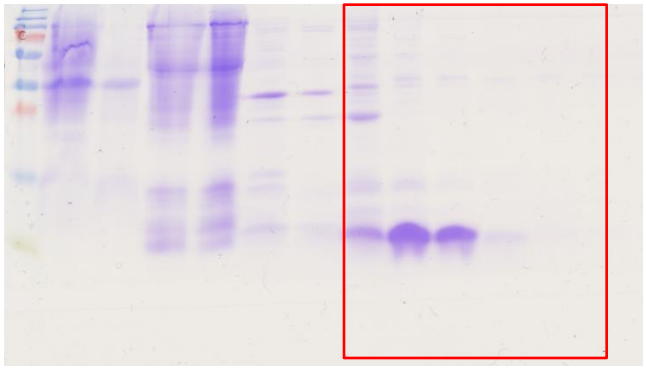
Protein only pH 8 (green) plus 2mM peptide (pink)

There is a specific interaction between HIV CTD integrase and the monomethylated H4K20 peptide, and these interactions are pH dependent

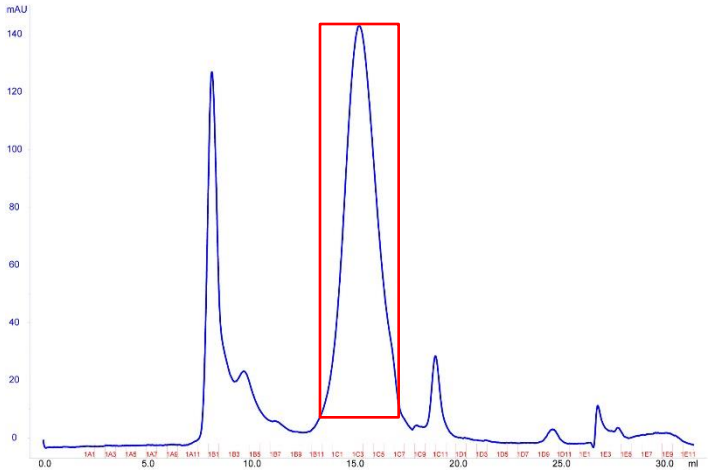
Purification of double labelled (^{15}N, ^{13}C) HIS-IN-CTD 220-270

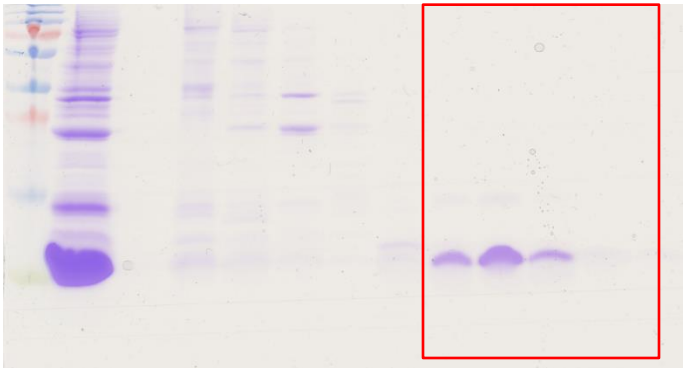
HIS-Trap FF crude



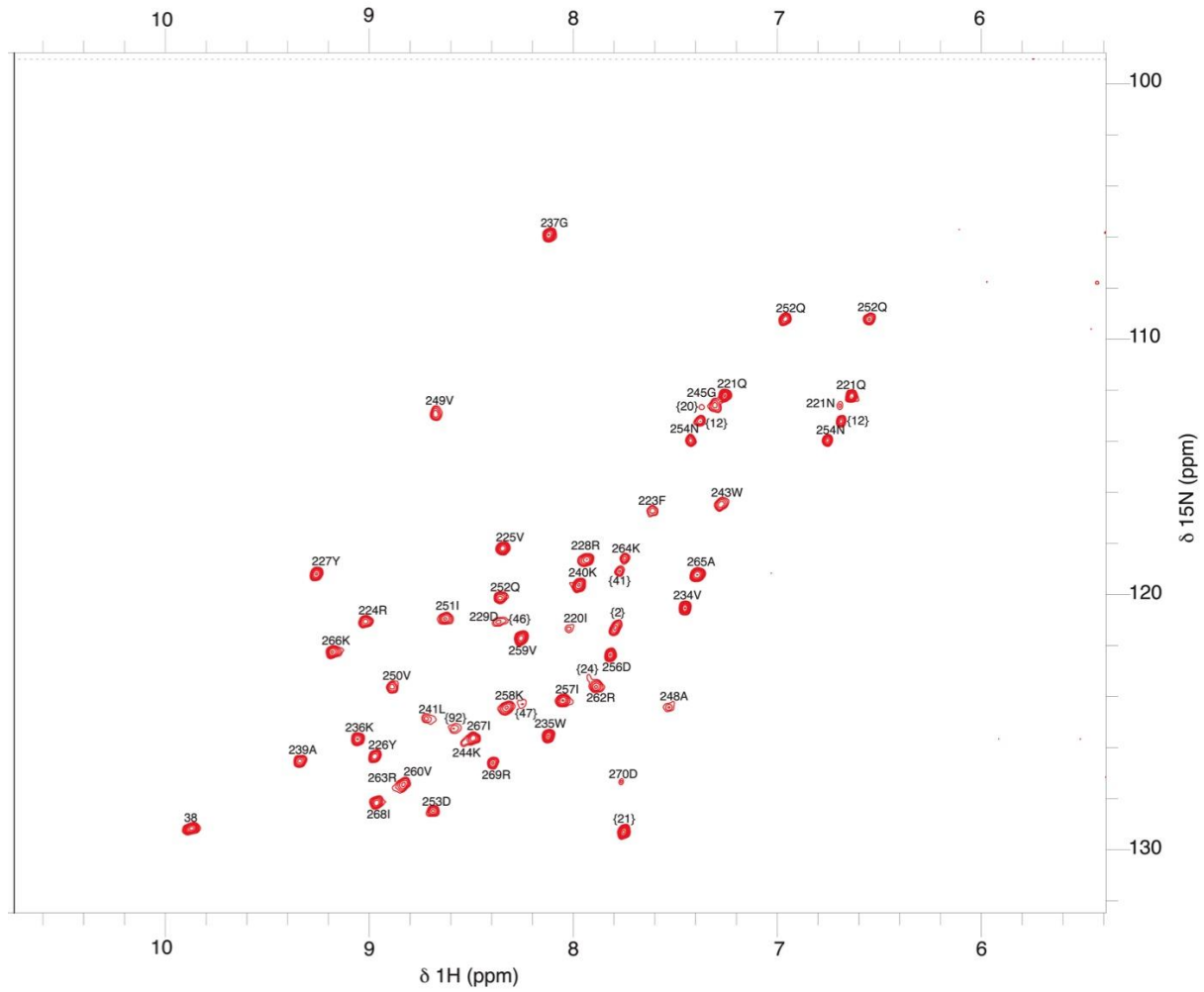


S75 16/60



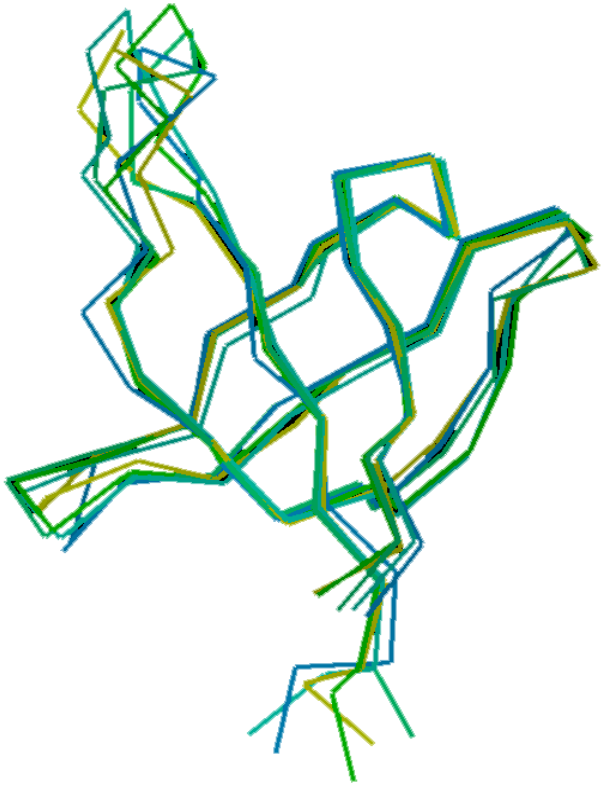


Residue Assignment of HIS-IN-CTD-220-270



Structure in progress

NMR structures of IN-CTD 220-270

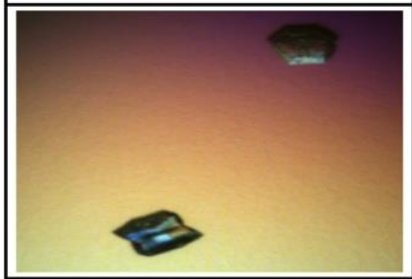
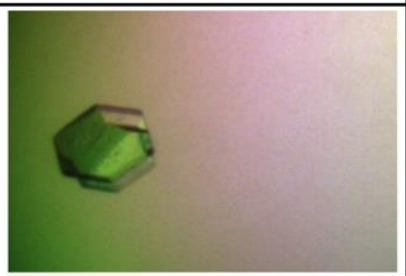
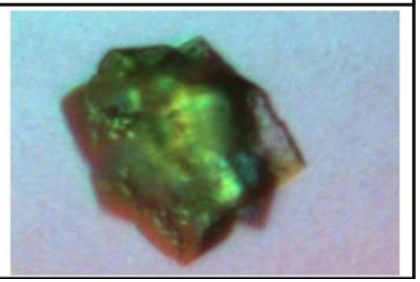


Quantification of chemical Shift Changes upon peptide addition

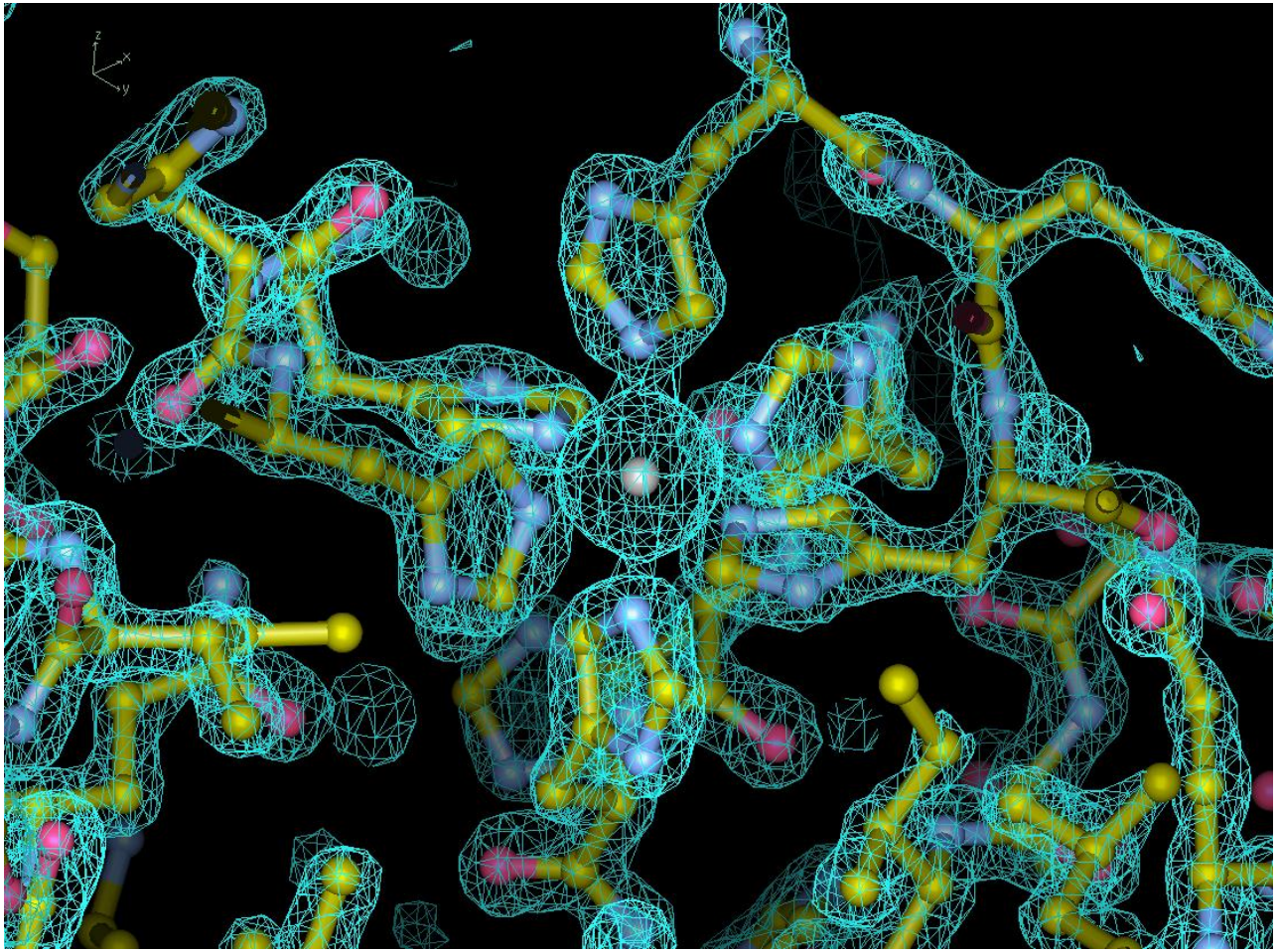
↓

Characterization of residues involved in the interaction between H4K20Me1 and IN-CTD

X-ray Structure of IN-CTD 220-270 at pH 7

0.01M MES pH5.6, 0.01M $MgCl_2$, 1M Lithium Sulfate	0.1M MES pH6.5 0.8M Sodium Malonate	0.1M Tris pH7, 1.5M Potassium Sodium Tartrate, 0.2M LiS04	0.1M Tris pH8.5, 1.26M$(NH_4)_2SO_4$, 0.2M Lithium Sulfate
			

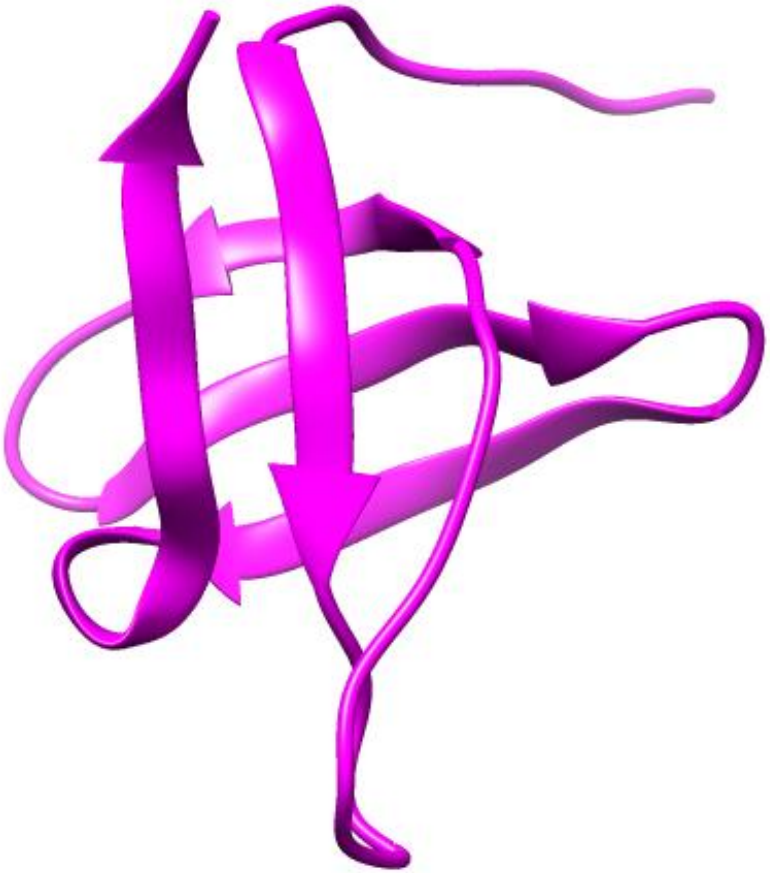
X-ray Structure of IN-CTD 220-270 at pH 7

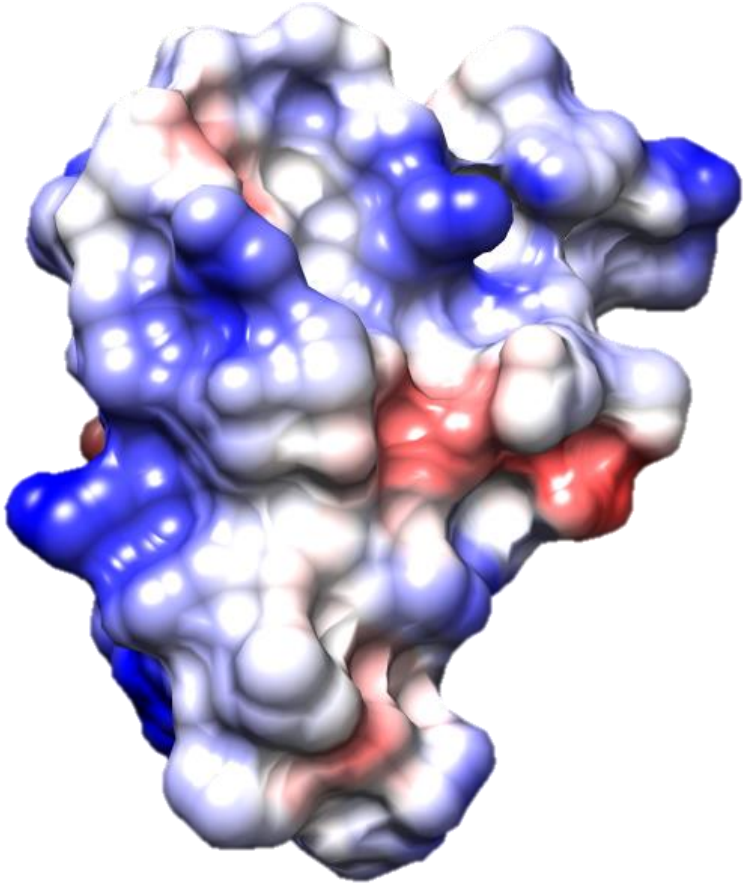


Resolution – 1.5Å
Space Group – $P6_3$
R-work – 0.1997
R-free – 0.2284

2Fobs-Fcalc at 2.3 σ

X-ray Structure of IN-CTD 220-270 at pH 7

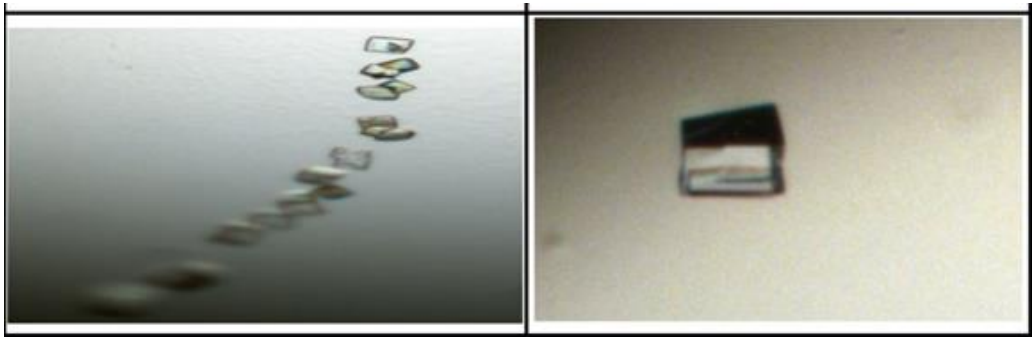


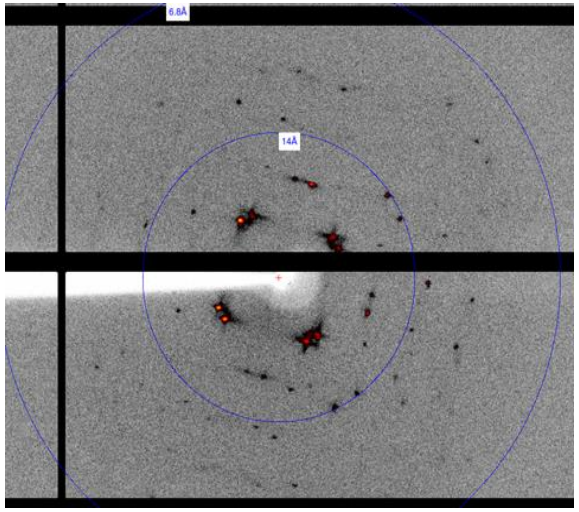


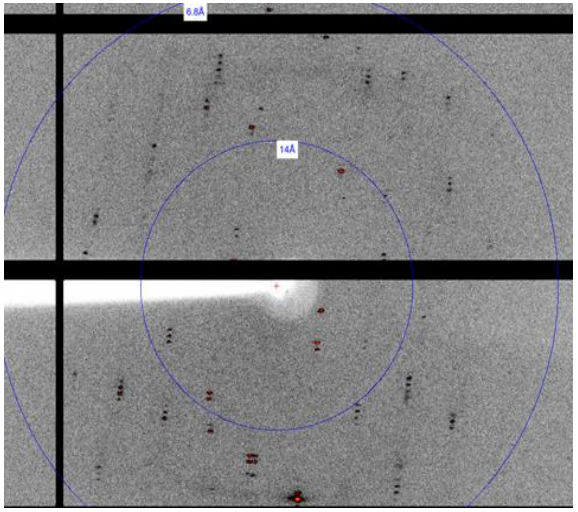
Blue – positive (basic residues)
Red – negative (acidic residues)
White –hydrophobic

IN-CTD 220-270 /peptide / DNA

Data at 4Å in the presence of DNA and peptide





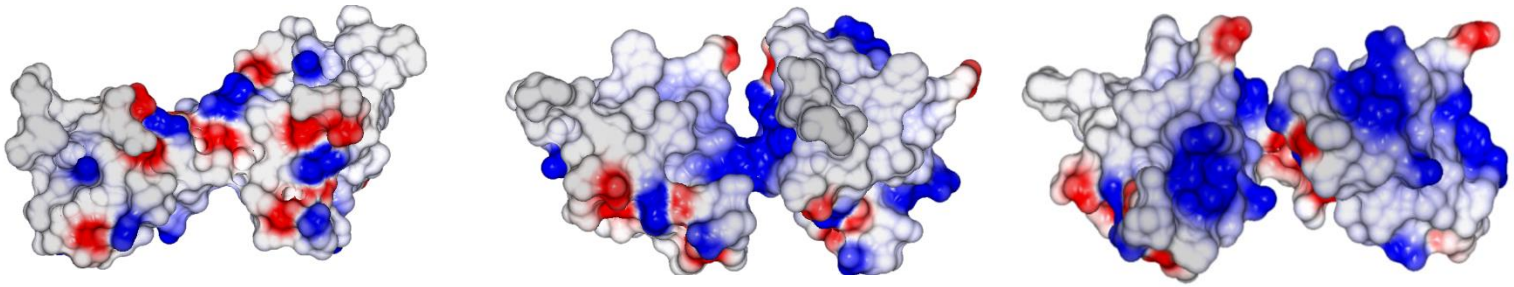


NMR, X-ray, CD data combination

Peptide Binding, multimerization, pH effects

- Deciphering of residues affected by peptide binding and participating in the peptide recognition site

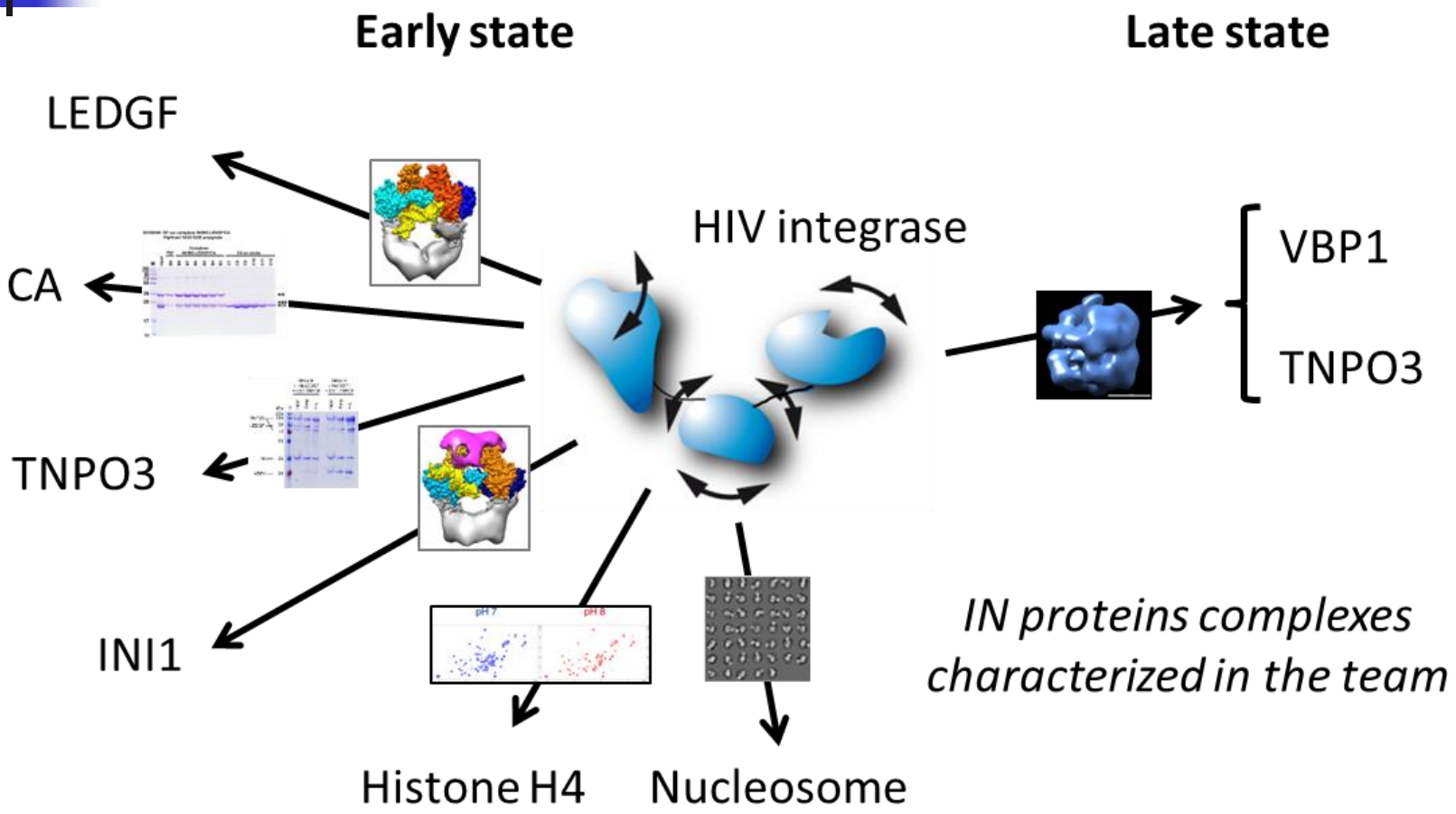
- Presence of 3 multimerization interfaces



- Structural changes induced by pH

Integration of functional and high resolution NMR and Crystallographic data with cryo-EM data → need for better cryo-EM structures

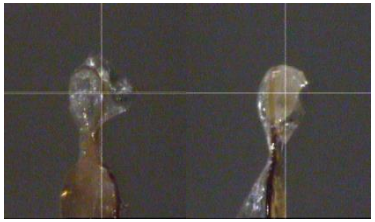
Characterized complexes

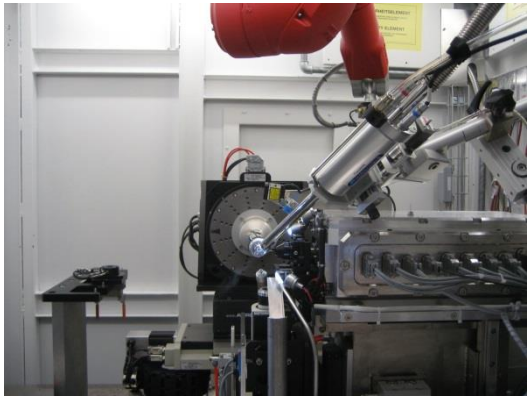


Complexes suitable for low resolution EM studies but not for high resolution and not for crystallization : too many flexible / unstructured parts

Towards high resolution structure

- **Increase solubility, stability and homogeneity**
  - Produce stable complexes in cell (Production without solubilizing agents (small detergents, high salt))
  - Effect of PTMs on stability (Setup efficient production in mammalian cells)
  - Others partners (full length, domains, ligands, nanobodies, …)
  - Complex reconstitution procedures
- **Improve crystallization setups**
  - Setup analysis procedures to decipher the best physico-chemical conditions (DNA stoechiometry, pH, ionic force…) for the stabilization of non-covalent complexes in homogeneous conformation (thermofluor, DLS, ITC)
  - Fast screening of crystals (in crystallization drop screening)

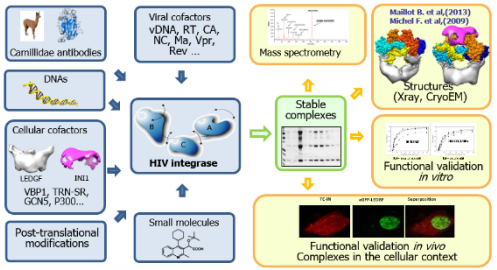




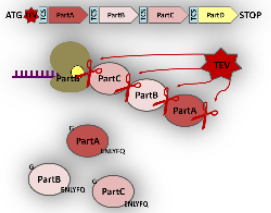
Methods development

Produce stable complexes: Production of unstable proteins through the formation of stable core complexes

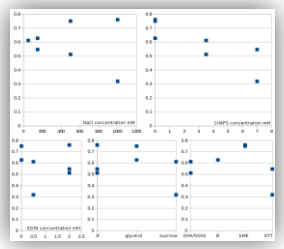
Levy N., Eiler S., et al. Nat Comm (2016); Eiler S., Levy N. et al, Methods Mol Biol (2017), in press.



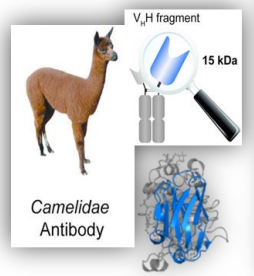
Produce stable complexes in cell: *In situ* protein complexes production in prokaryotic and eukaryotic cells



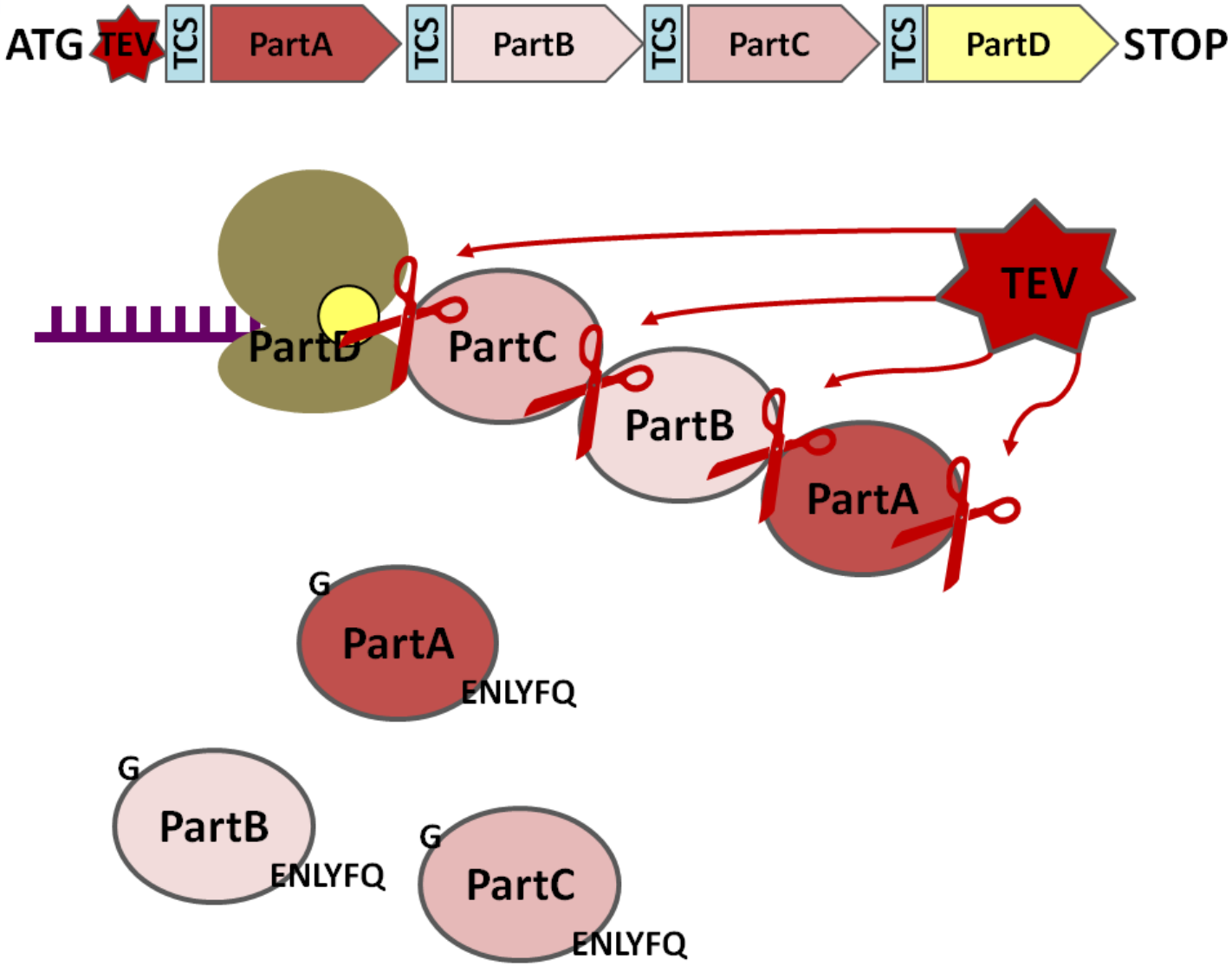
Decipher the best physico-chemical conditions: setup a sparse matrix approach for proteins/complexes stability and solubility optimization



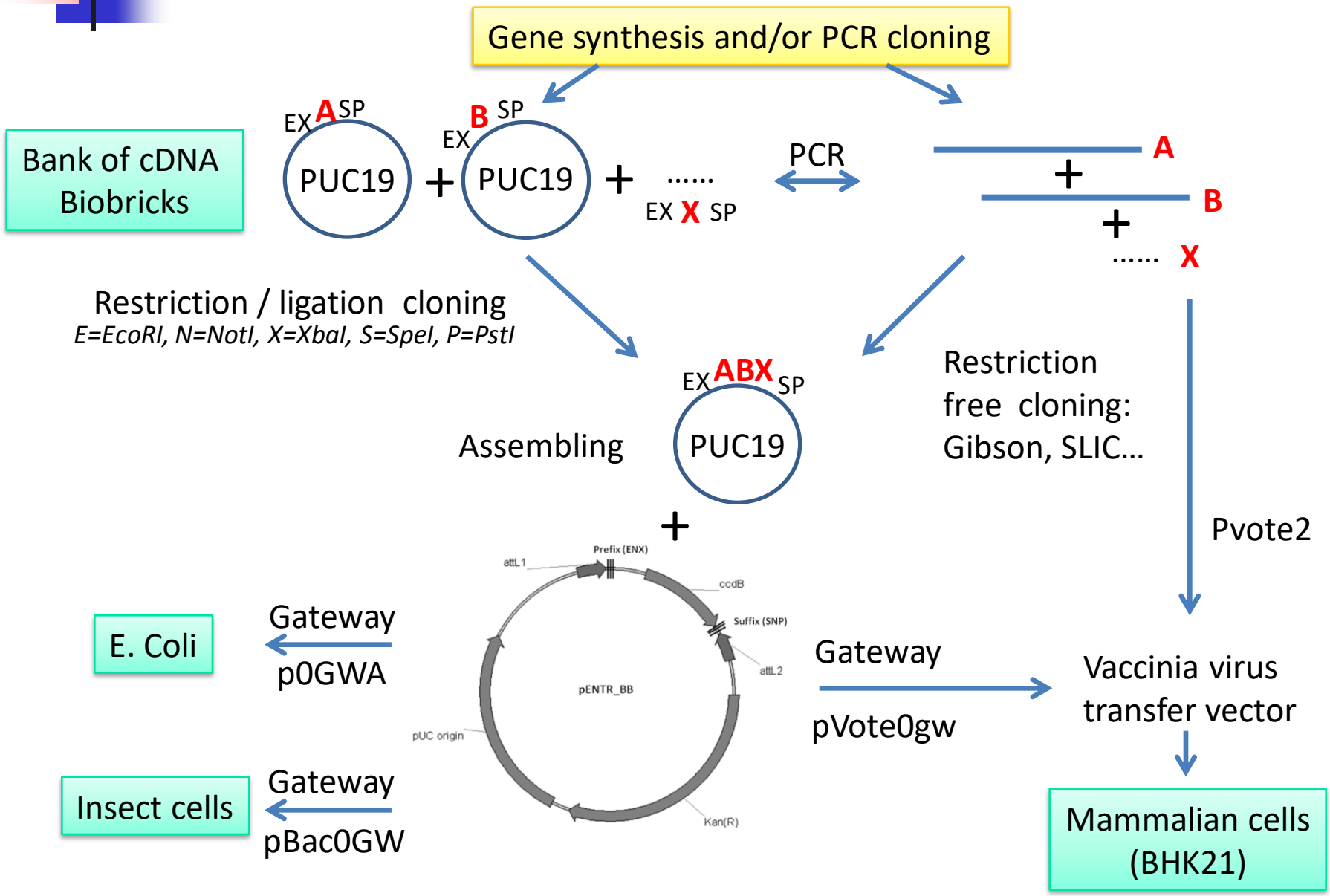
Other partners: Disordered protein stabilization by nanobodies (Alain Roussel)



Protein complexes expression in E. Coli, Insect and mammalian cells



Protein complexes expression in E. Coli, Insect and mammalian cells

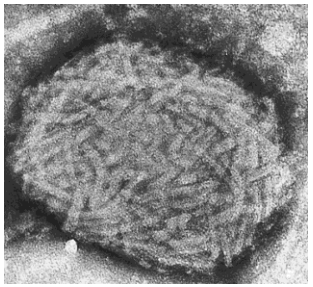


Biobrick collection for protein expression in E. Coli, Insect and mammalian cells

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
	TRNSR2	Human Transportin
	TT8-eYFP	Twin TEV cleavage site 8 – enhanced Yellow Fluorescent Protein
	……	

IN/LEDGF in mammalian cells (vaccinia virus)

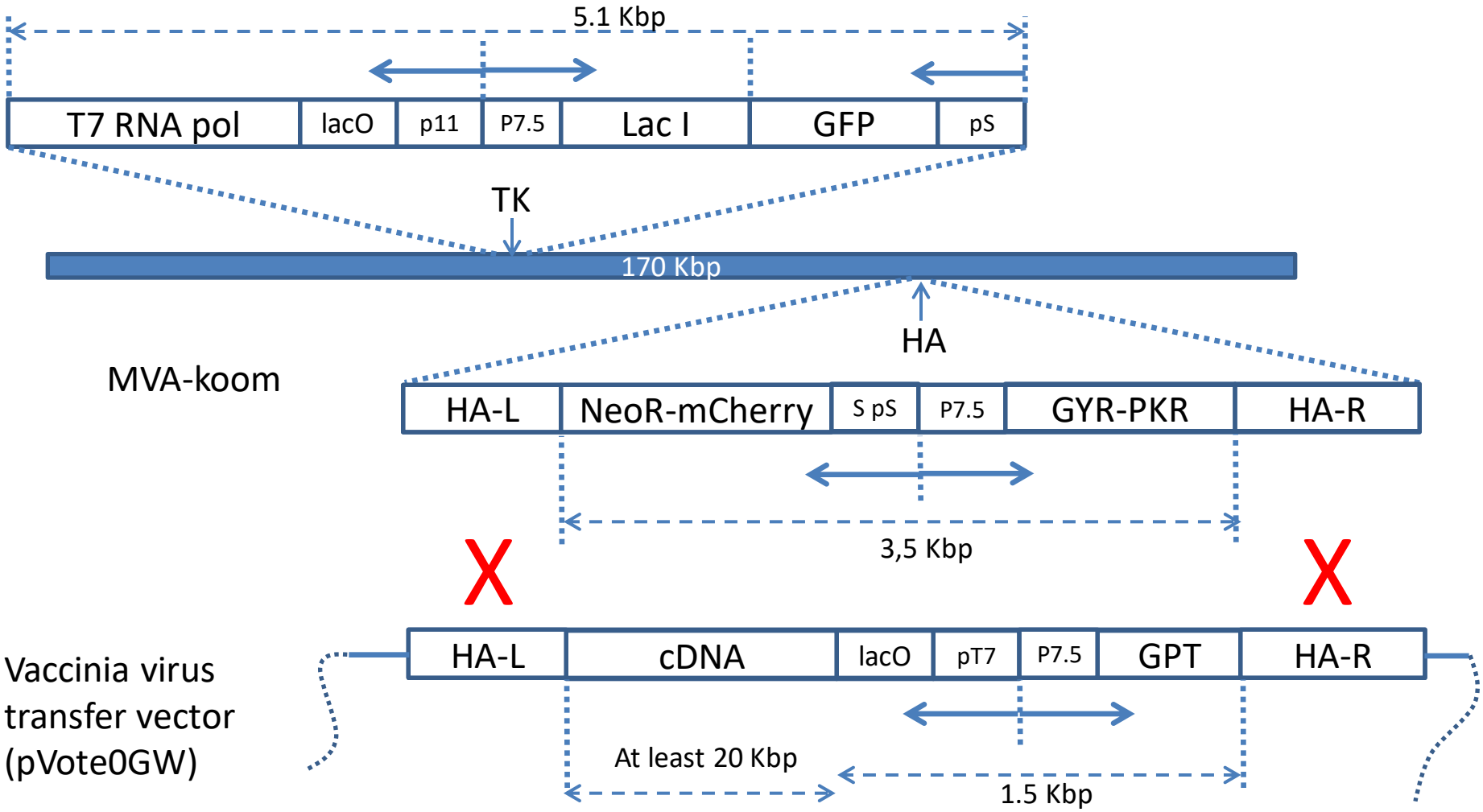
- **Vaccinia virus**: poxvirus family
  - dsDNA virus (≈ 200 kb) encoding its own transcription and replication machinery
  - viral multiplication in the cell cytoplasm: no RNA splicing
  - viral infection diverts the cellular machinery in its favour
  - at least 25 kb of foreign DNA



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

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

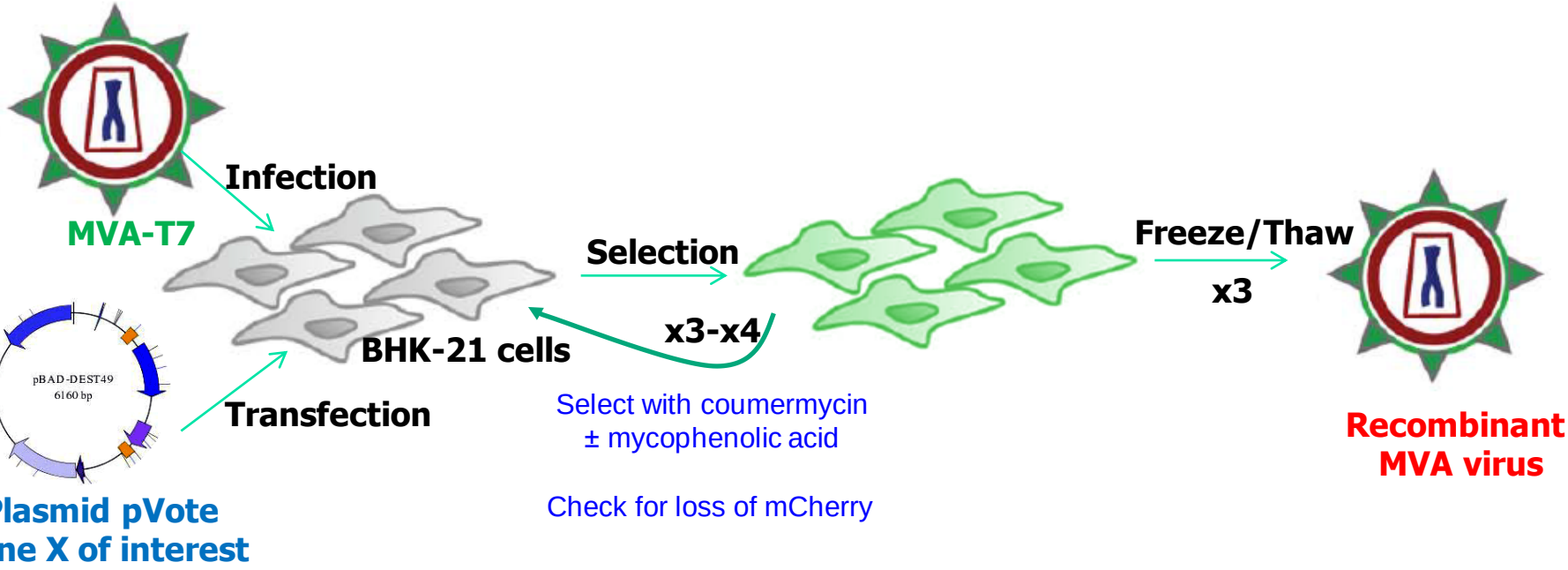
- **Encode T7 RNA polymerase, IPTG inducible**



Before recombination: GFP(+), mCherry(+), NeoR(+), GYR-PKR(+) (Coumermycin Sensible), GPT(-) (Mycophenolic acid sensible)

After recombination: GFP(+), mCherry(-), NeoR(-), GYR-PKR(-) (Coumermycin Resistant), GPT(+) (Mycophenolic acid resistant)

Recombinant MVA selection



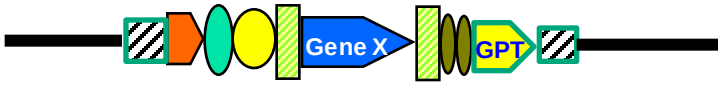
Parental virus genome

Negative selection: (monitored by mCherry loss) **+ coumermycin**

=> Gyrase dimerisation => active PKR => eif2α phosphorylation

=> translation inhibition => cell death

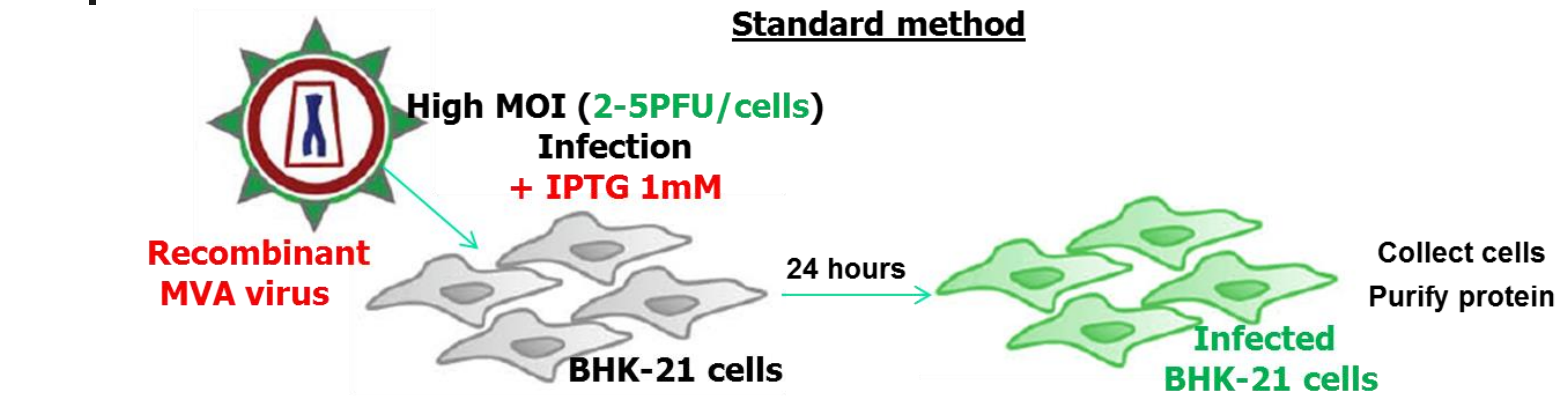
Recombinant virus genome

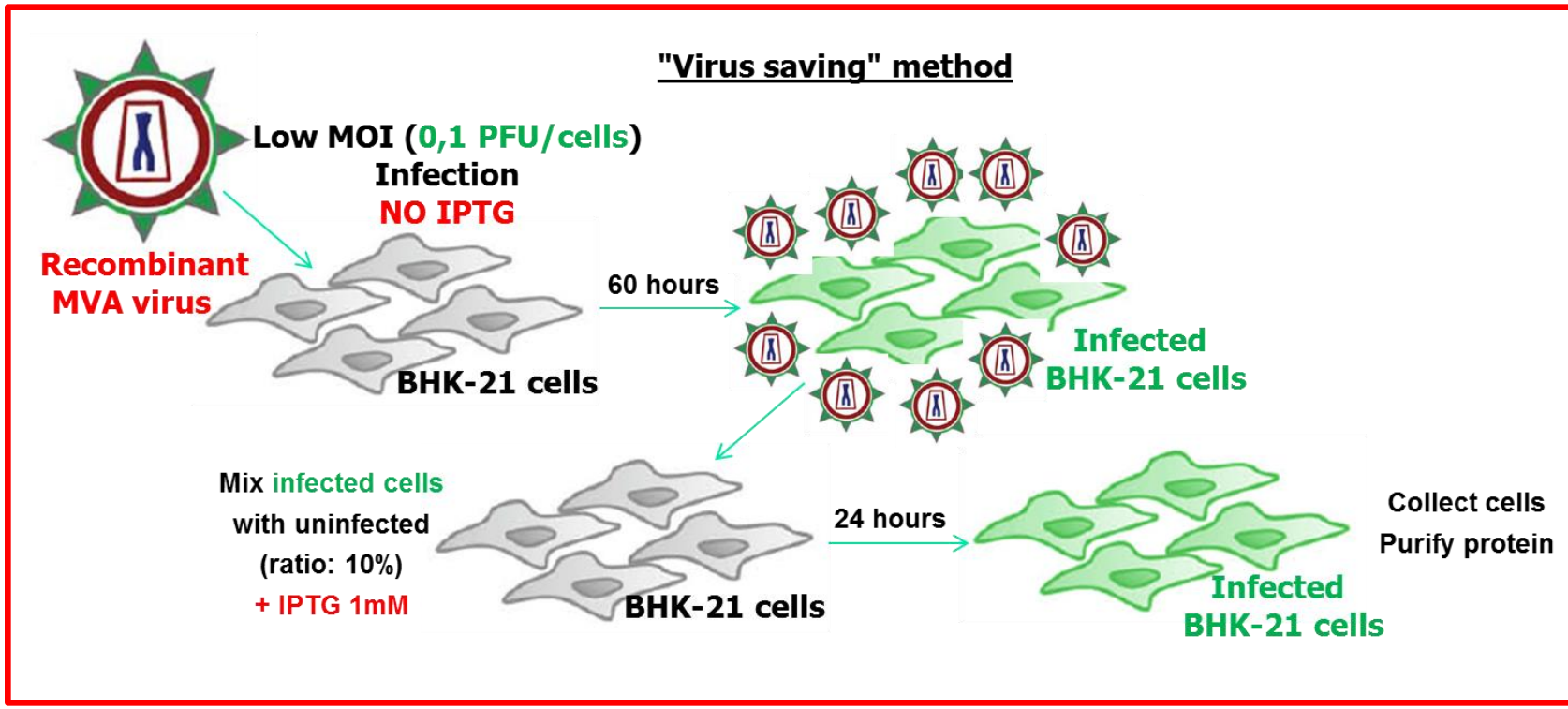


Positive selection: **+ mycophenolic acid**

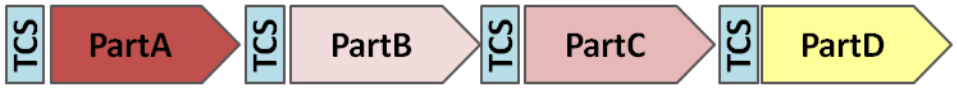
=> GPT integration selection

MVA Infection methods





Mammalian cell protein complexes production



Vaccinia virus transfer vector pVote2

+

Parental virus MVA-T7-Koom

+mycophenolic acid
(select the recombinant virus)

+ coumermycin
(kills cells with parental virus)

Recombinant virus

15-20 days

500 ml
10^6 Cell/ml

Infect **0.1 PFU/cell**
no IPTG

500 ml BHK21 mammalian cells (10^6 Cells/ml)

250 ml BHK21 mammalian cells (10^6 Cells/ml)

48 hours

5500 ml BHK21 mammalian cells (10^6 Cells/ml)

+ IPTG

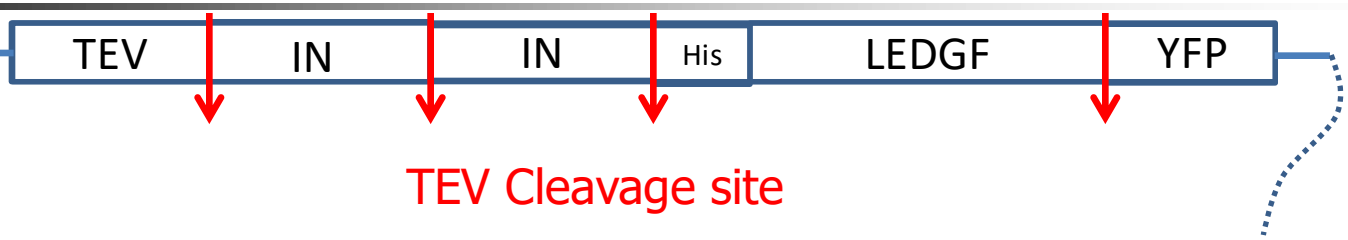
5000 ml BHK21 mammalian cells (10^6 Cells/ml)

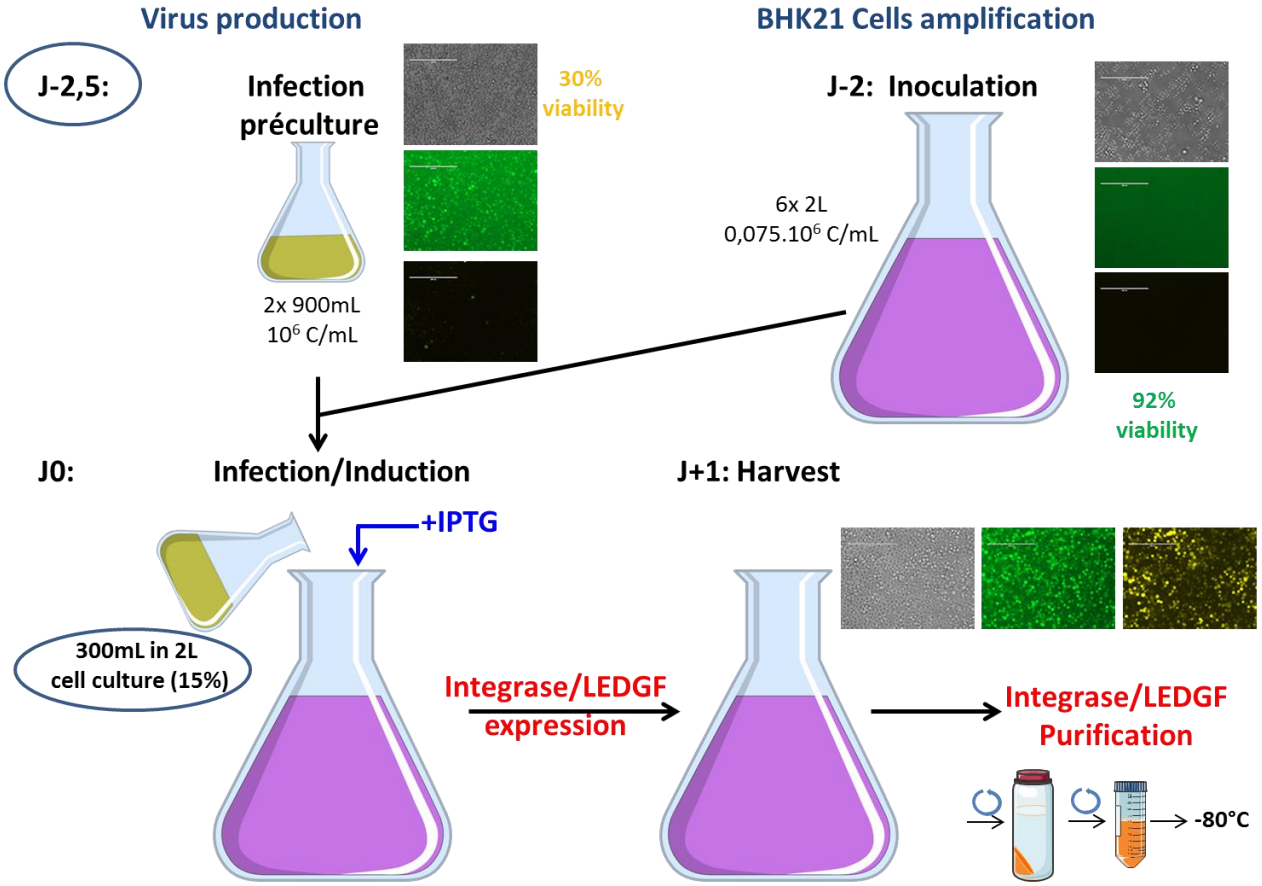
24 hours

Collect cells and purify protein

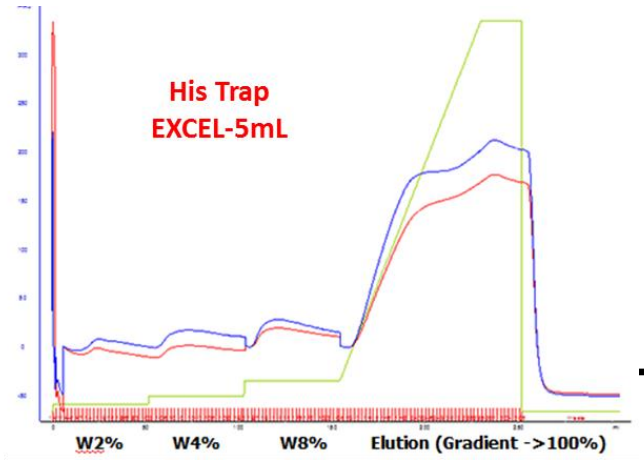
7-10 days

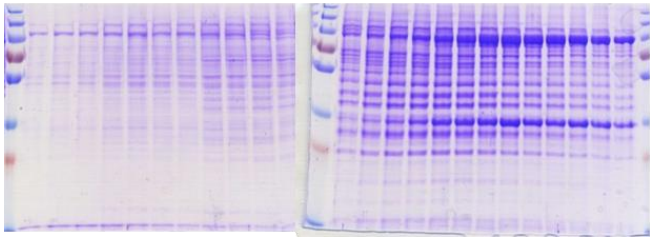
Large scale production of Integrase/LEDGF complex in mammalian cells



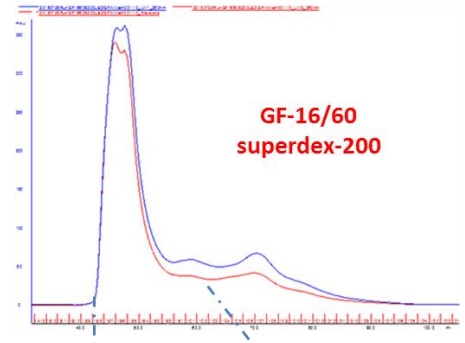


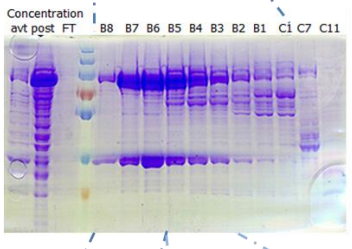
Large scale production of Integrase/LEDGF complex in mammalian cells





Concentration
on Amicon 100Kd
to a volume of 4,5mL



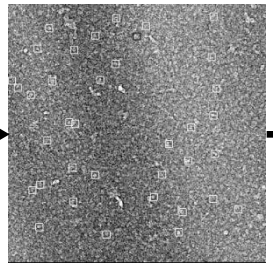


"pure" fractions 1,1mg/mL 6,5mg

"contaminated" fractions 0,6mg/mL 3,5mg

Total= 10mg of complex

(IN/LEDGF) mam / DNA complex →

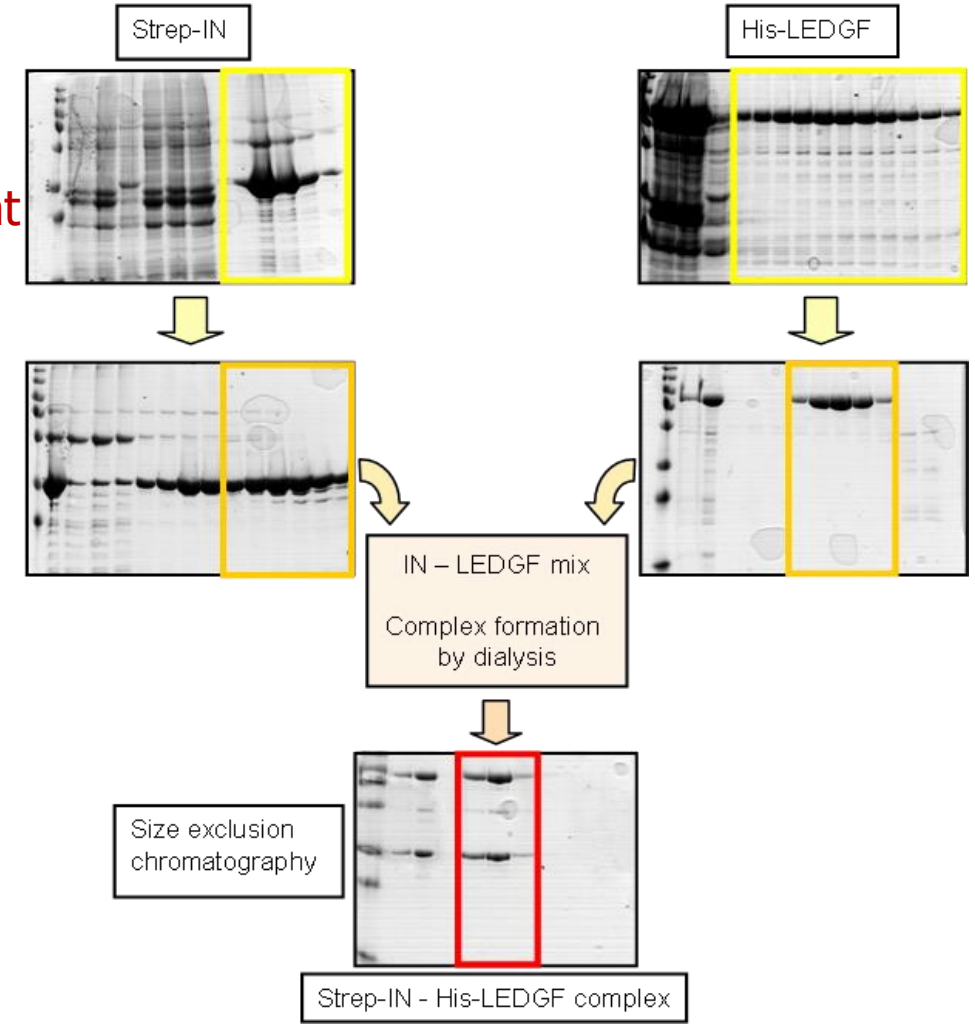


→ In progress

Comparison of HIV-1 IN produced in E. Coli, Insect and mammalian cells

IN/LEDGF : complex formation and purification in Insect cells

Expression vector: Baculovirus



High salt, detergent

150 mM NaCl
7mM CHAPS

Affinity column

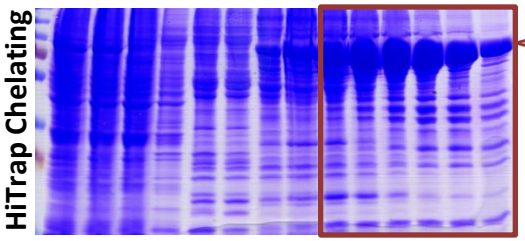
Gel filtration

150 mM NaCl

IN/LEDGF complex production in mammalian cells: mono expression

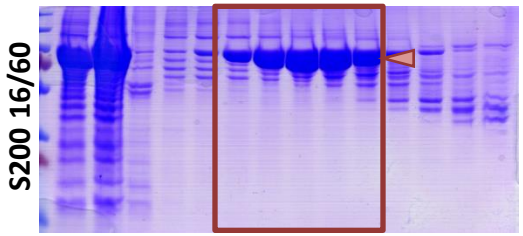
His-LEDGF
Mammalian suspension cells
Lysis, sonication, centrifugation
→ Soluble extract

Affinity chromatography



5-10mg / 10^9 cells (1L)

Gel filtration



LEDGF

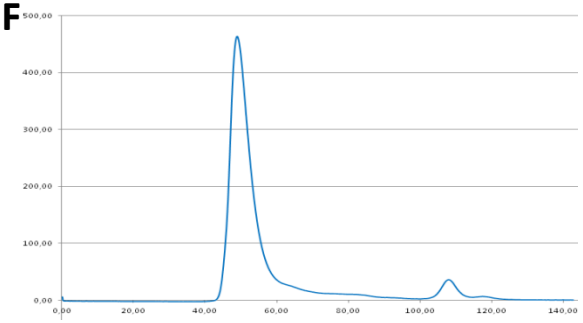
Mix
LEDGF + IN

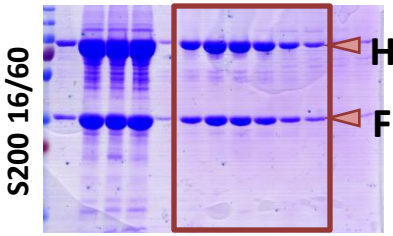
7mM CHAPS → 0mM

1M NaCl → 0,25M

Complex formation by dialysis

Gel filtration



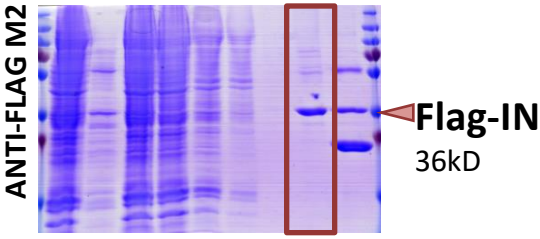


3mg/L of mammalian cells

IN-LEDGF complex

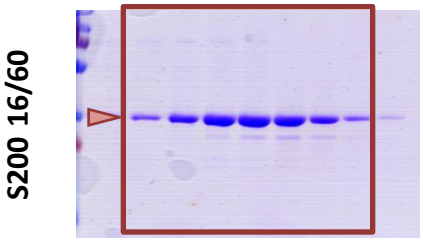
FLAG-IN
Mammalian suspension cells
Lysis, sonication, centrifugation
→ Soluble extract

Affinity chromatography



8mg / 10^9 cells (1L)

Gel filtration



IN

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

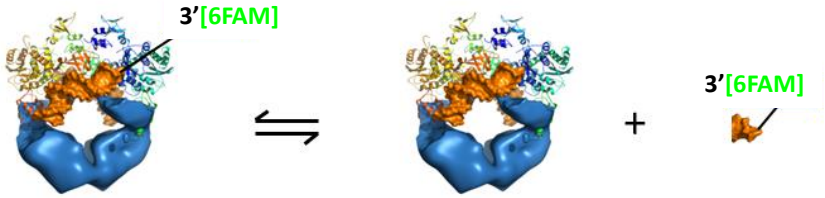
Solubility analysis

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

Solubility increase for IN produced in mammalian cells

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

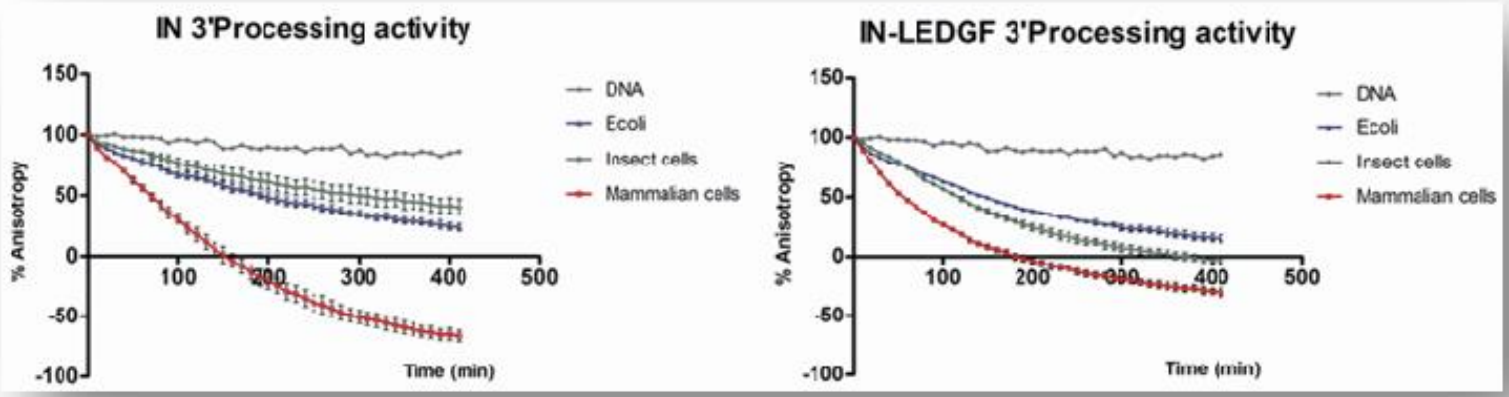
3′ processing IN activity by fluorescence anisotropy measurements



Slow rotation
High anisotropy

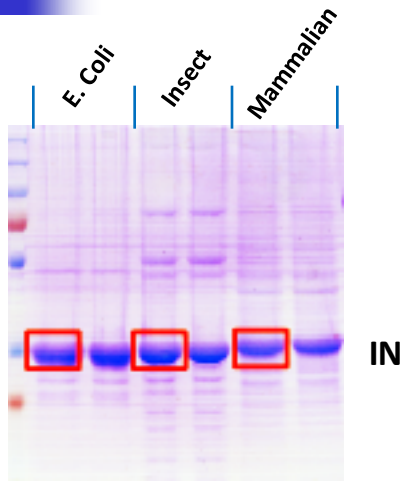
Fast rotation
Low anisotropy

5′-GACTACGGTTCAAGTCAGCGTGTGGAAAATCTCTAGCAGT-3′ [6FAM]
3′-CTGATGCCAAGTTCAGTCGCACACCTTTTAGAGATCCTCA-5′

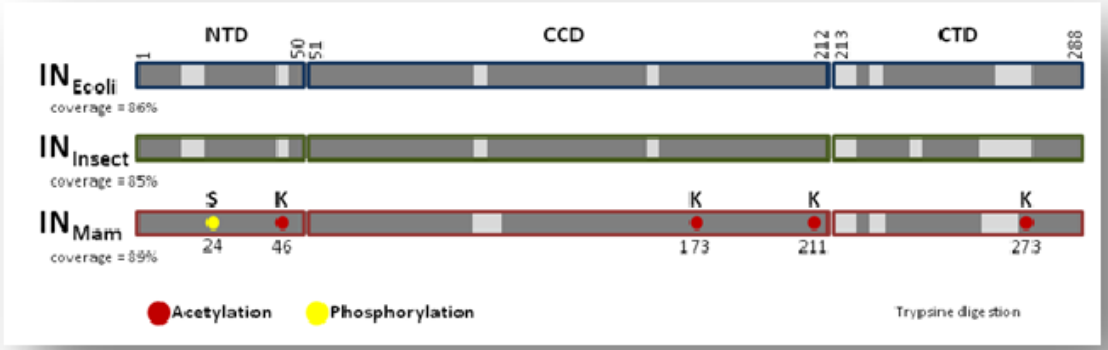


Increase of the 3′ processing activity for IN produced in mammalian cells

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



Post translational modification



S24, K46, K173, K211, K273

Band Excision

Reduction (DTT)
Alkylation (iodoacetamide)
Digestion (Trypsin, AspN)

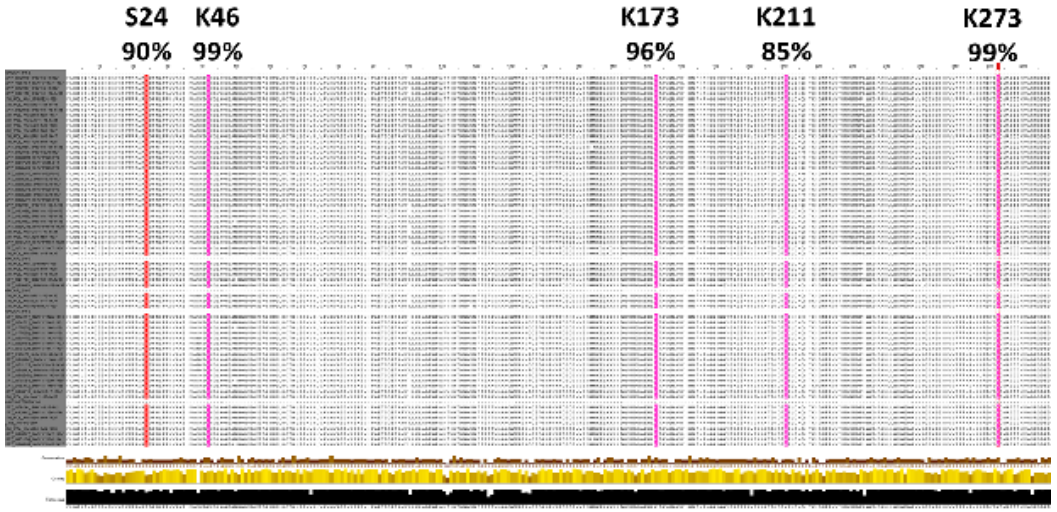
LTQ XL ETD

(Thermo Fisher Scientific)

nanoLC-nanoESI-CID/MS-MS

nanoLC-nanoESI-ETD/MS-MS

Sequence conservation

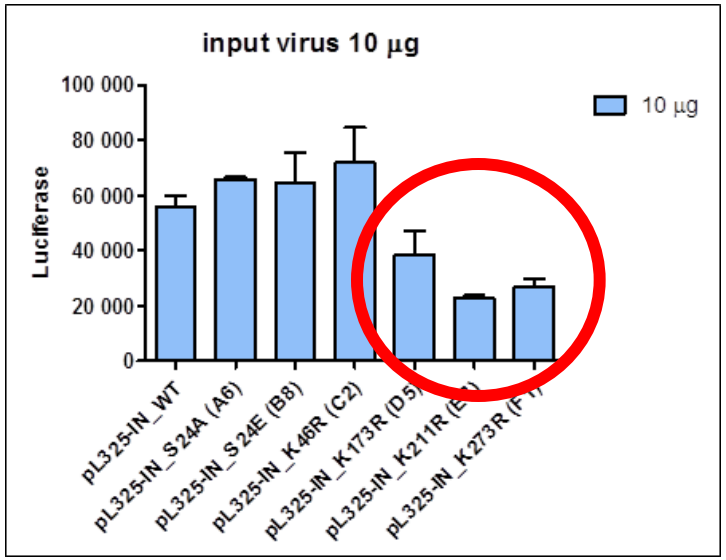


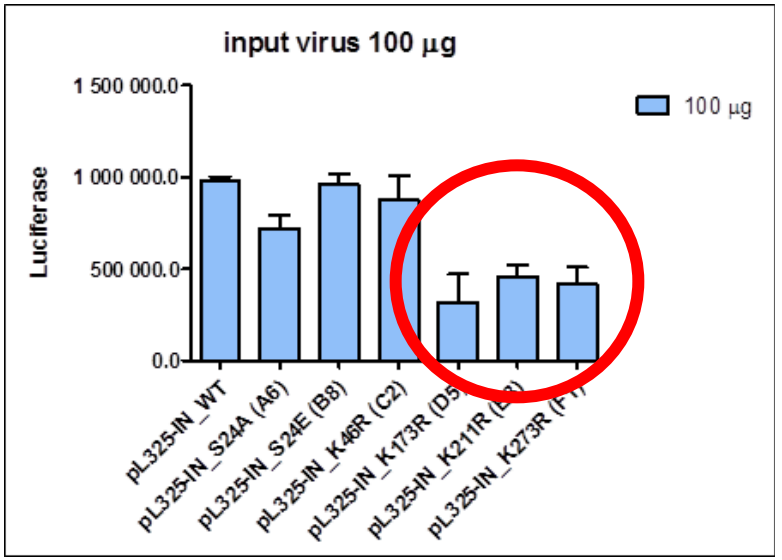
Phosphorylation S24, Acetylation K46, K173, K211, K273

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

THP1 cells infection with IN wild type and IN mutants virus

S24A, S24E, K46R, K173R, K211R, K273R



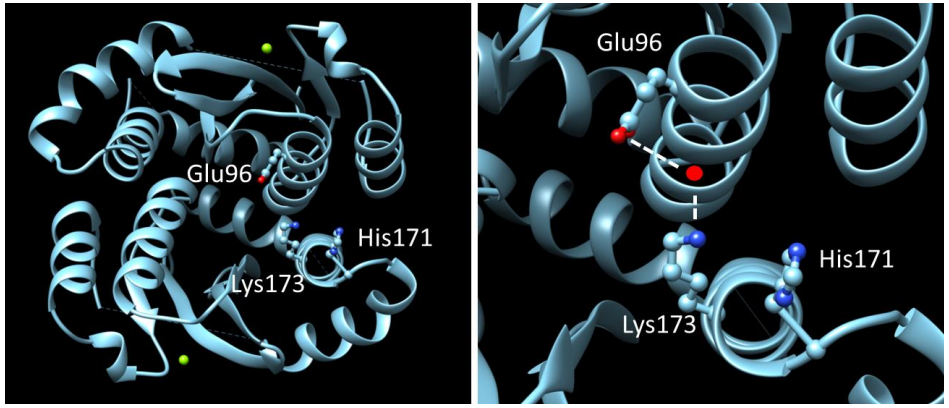


Lys --> Arg mutations: effect on viral replication for K173, K211 and K273

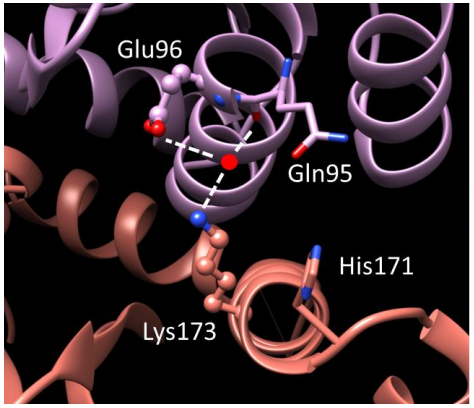
K173R structural analysis

Structural analysis of HIV-1 IN K173 acetylation

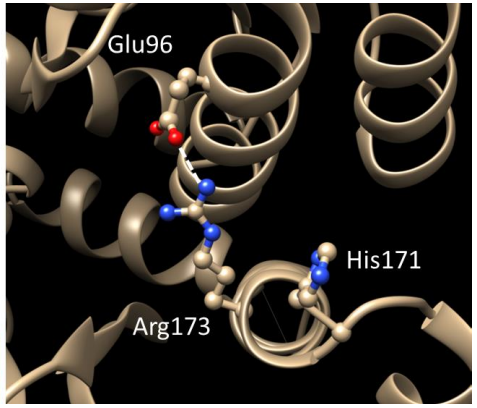
HIV-1 IN CCD X-ray structure:
K173 involved in the dimer stabilization



HIV-1 IN CCD + IN/LEDGF
interaction inhibitor X-ray Structure

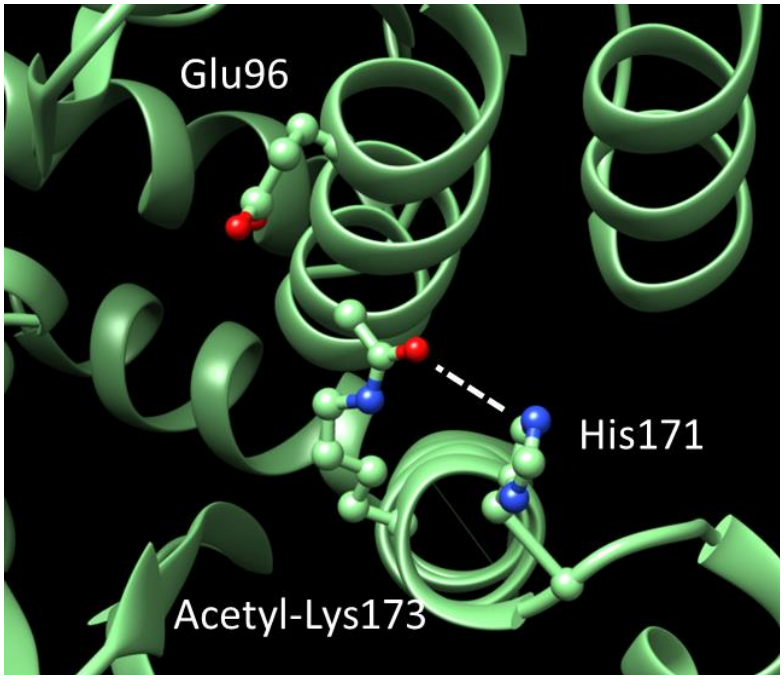


HIV-1 IN CCD K173R
X-ray Structure



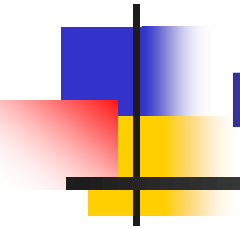
Stabilization of the HIV-1 IN dimer

HIV-1 IN CCD K173-NAcetyl
Model



Destabilization of the HIV-1 IN dimer

K173 acetylation weaken the IN - IN interaction in the dimeric interface resulting in increased flexibility and structural adaptability

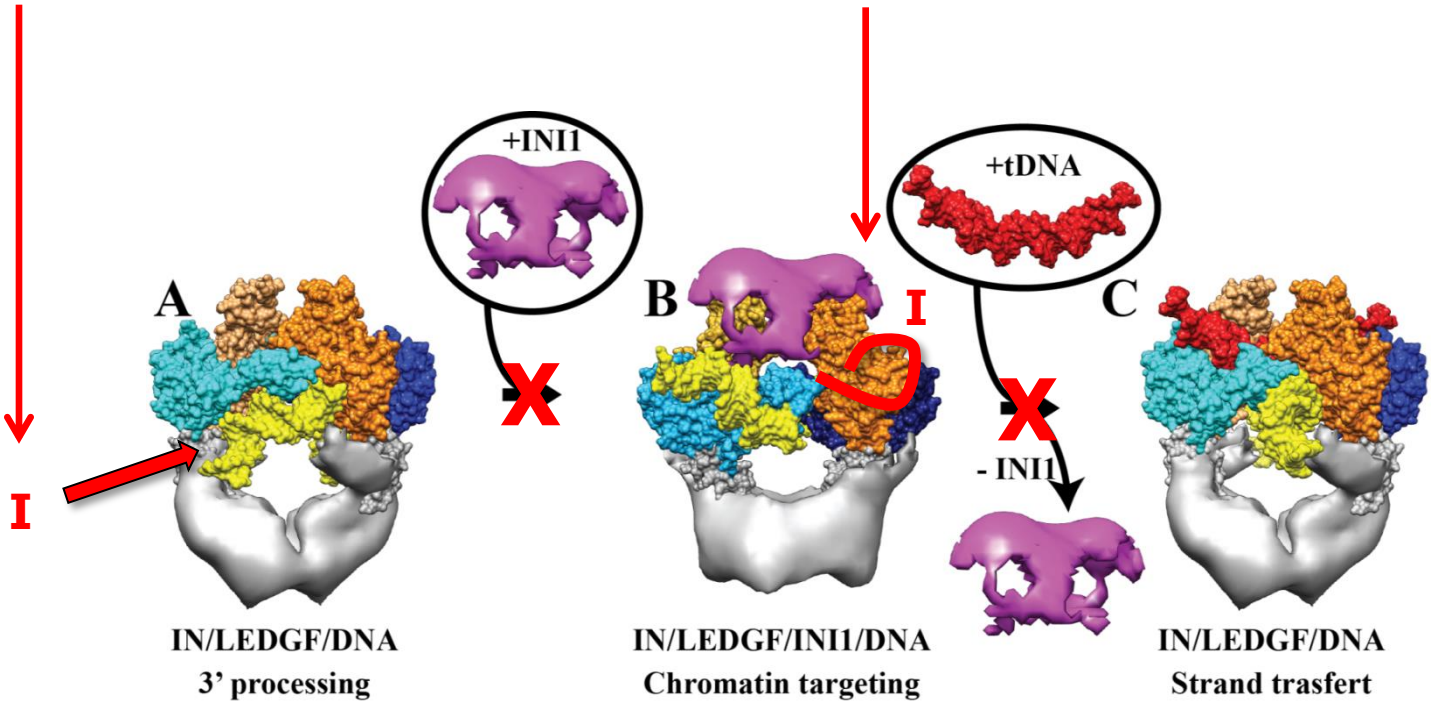


DRUG-DESIGN

Protein – Protein interaction and allosteric inhibitors

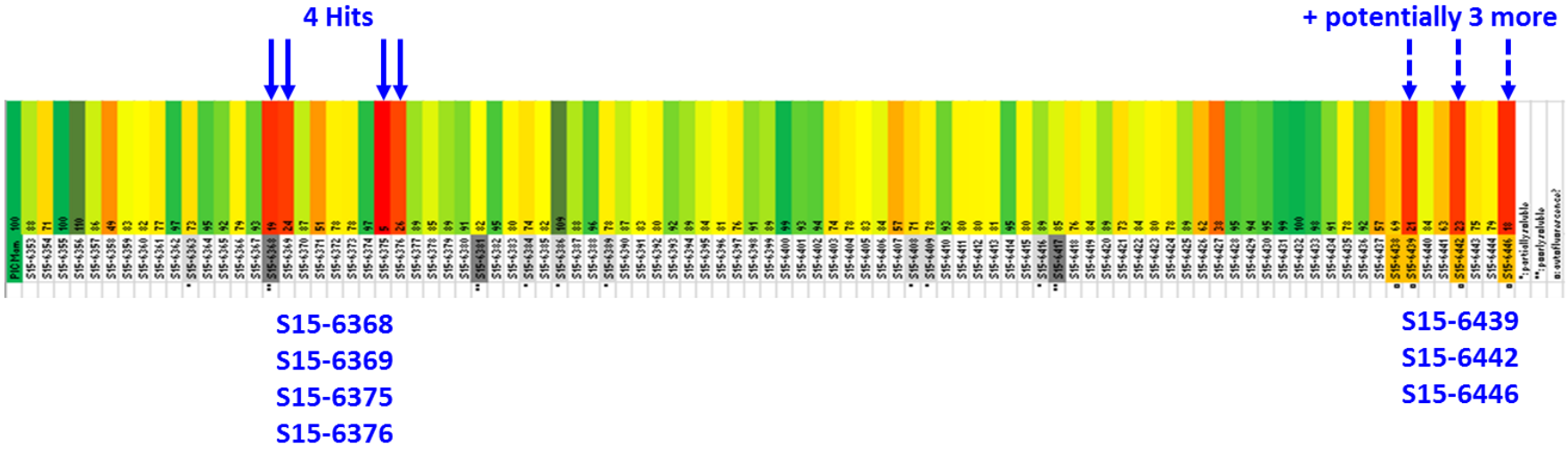
IN/LEDGF interaction inhibitors
Mutabilis

IN/LEDGF allosteric inhibitors
Shionogi



IN/LEDGF allosteric inhibitors

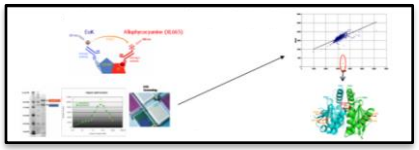
3’ processing assay adapted to 96 well plates, screening of a Shionogi sub library

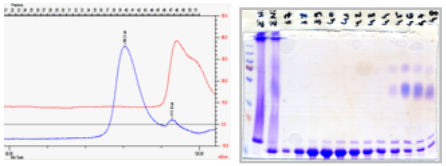


7 potential hits

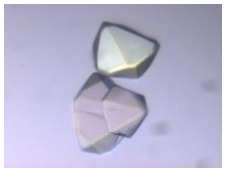
IN/LEDGF Protein – Protein interaction inhibitors

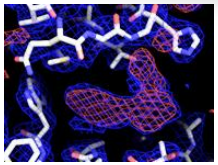
HTP IN – LEDGF interaction inhibitors screening (HTRF)





IN CCD Production and crystallization

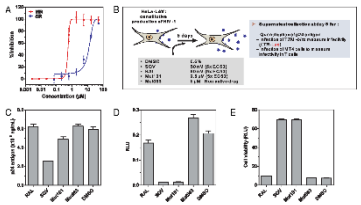




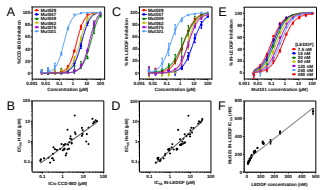
HTP inhibitors crystal soaking, data collection, structure

~100 structures solved

Structure based drug design

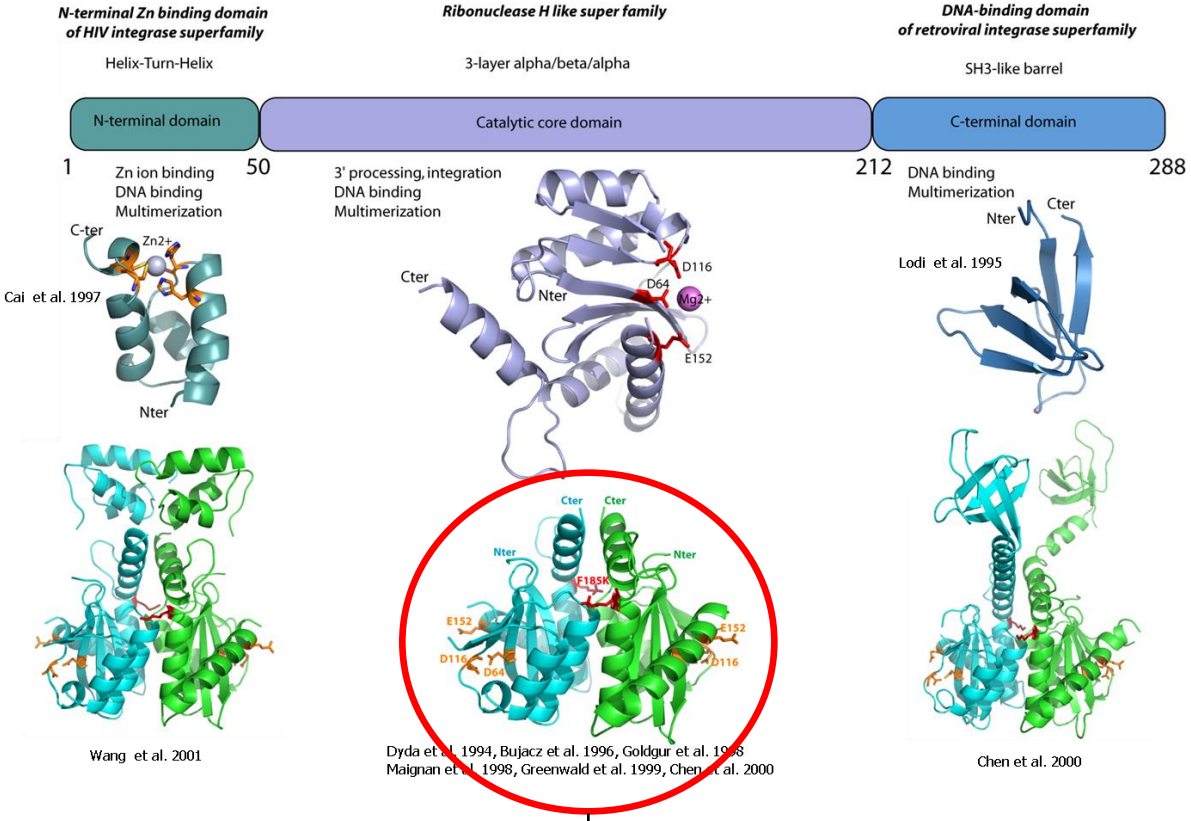


in-vitro functional assay



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

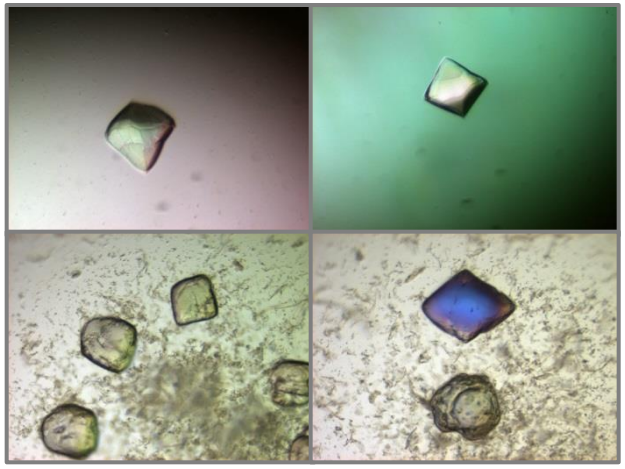
↓

Size exclusion chromatography
Superdex 200

↓

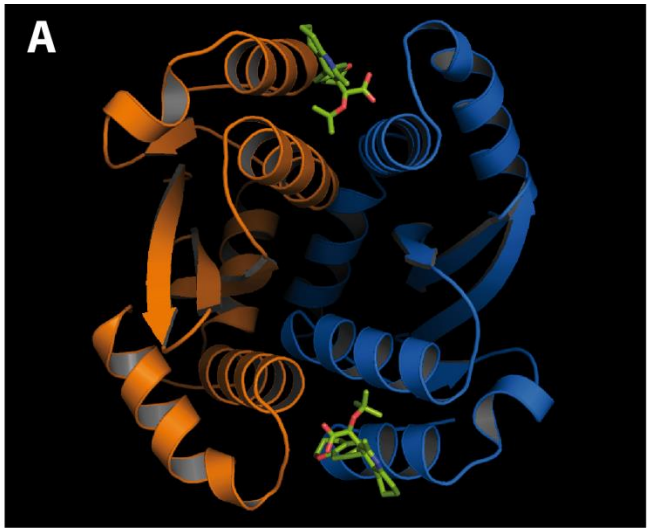
Crystallization
1.26 M AmSO4
100 mM NaCacodylate pH 6.5
Hanging drops
@ 22-23 °C

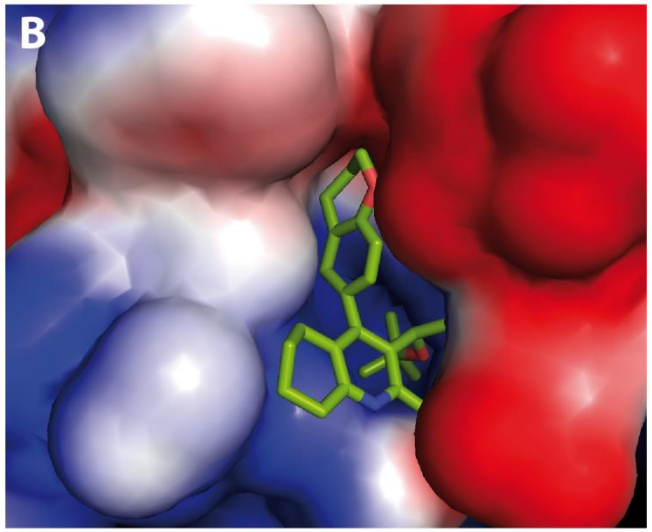
22-23°C
Diffraction
~2Å

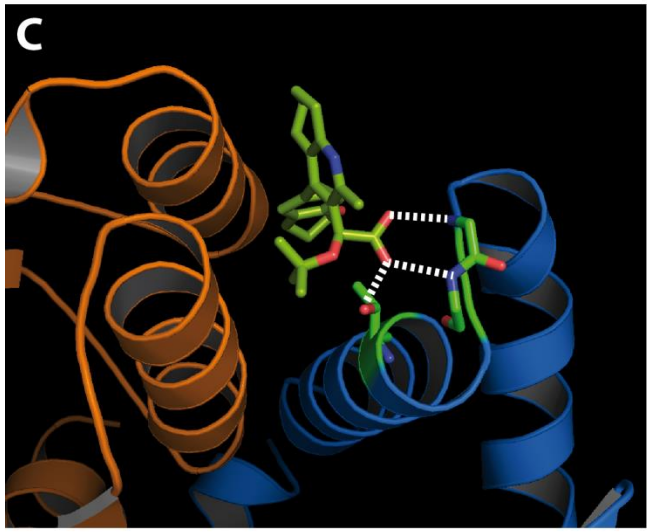


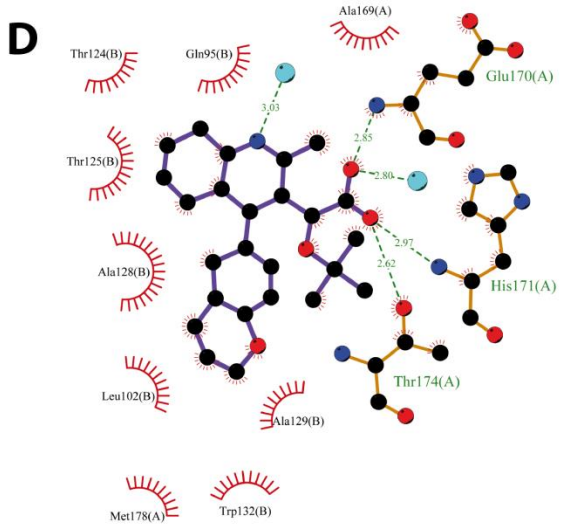
24-25°C
No diffraction !

HIV Integrase CCD – ligand structure



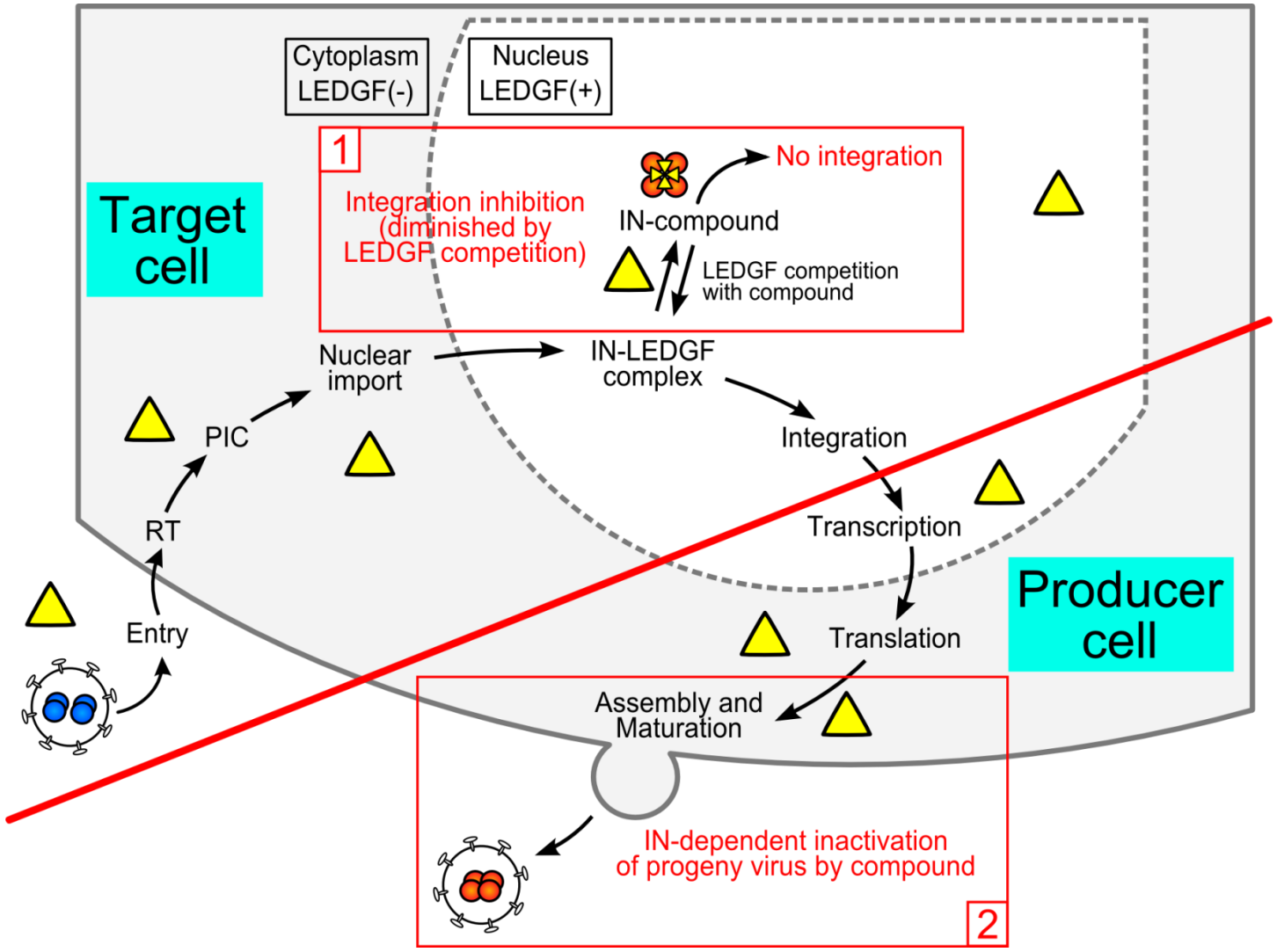




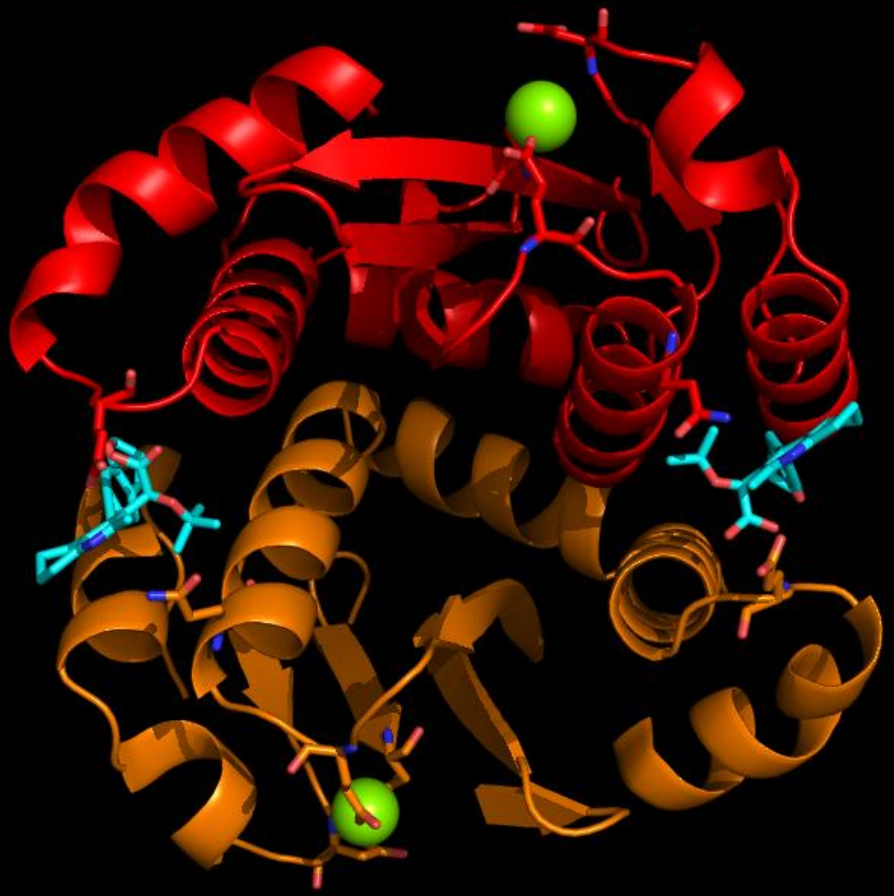


Le Rouzic et al., (2013), Retrovirology, 10, 144

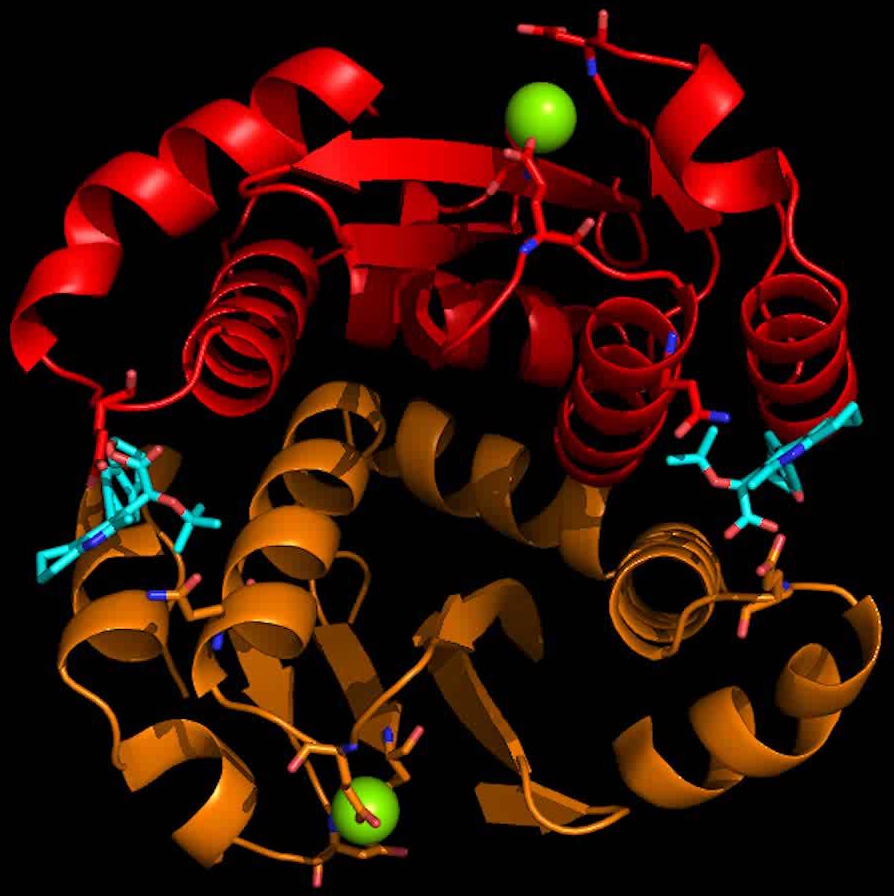
IN – LEDGF interaction and IN allosteric inhibitors



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



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



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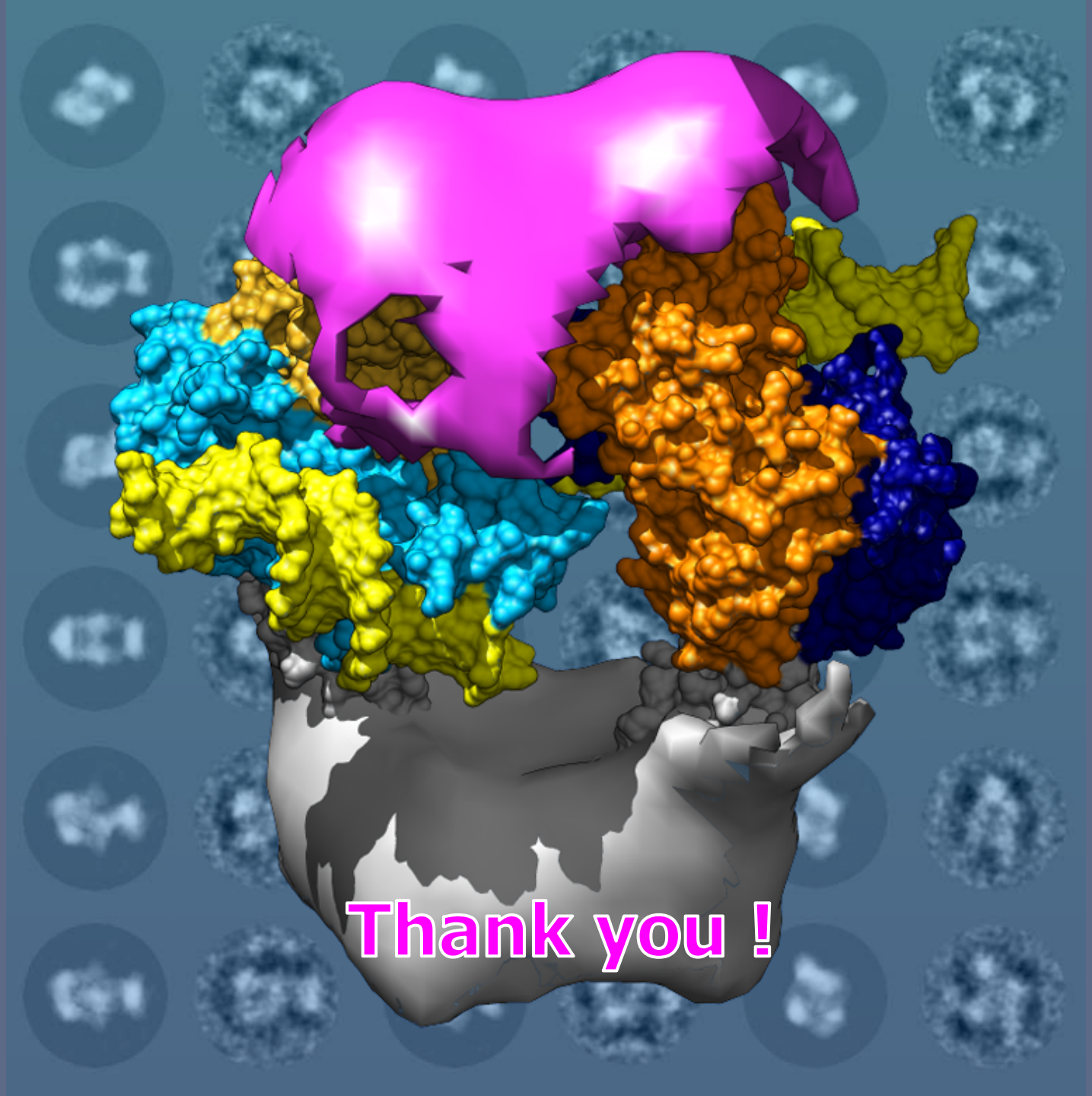
IGBMC common services and platforms


Thank you !