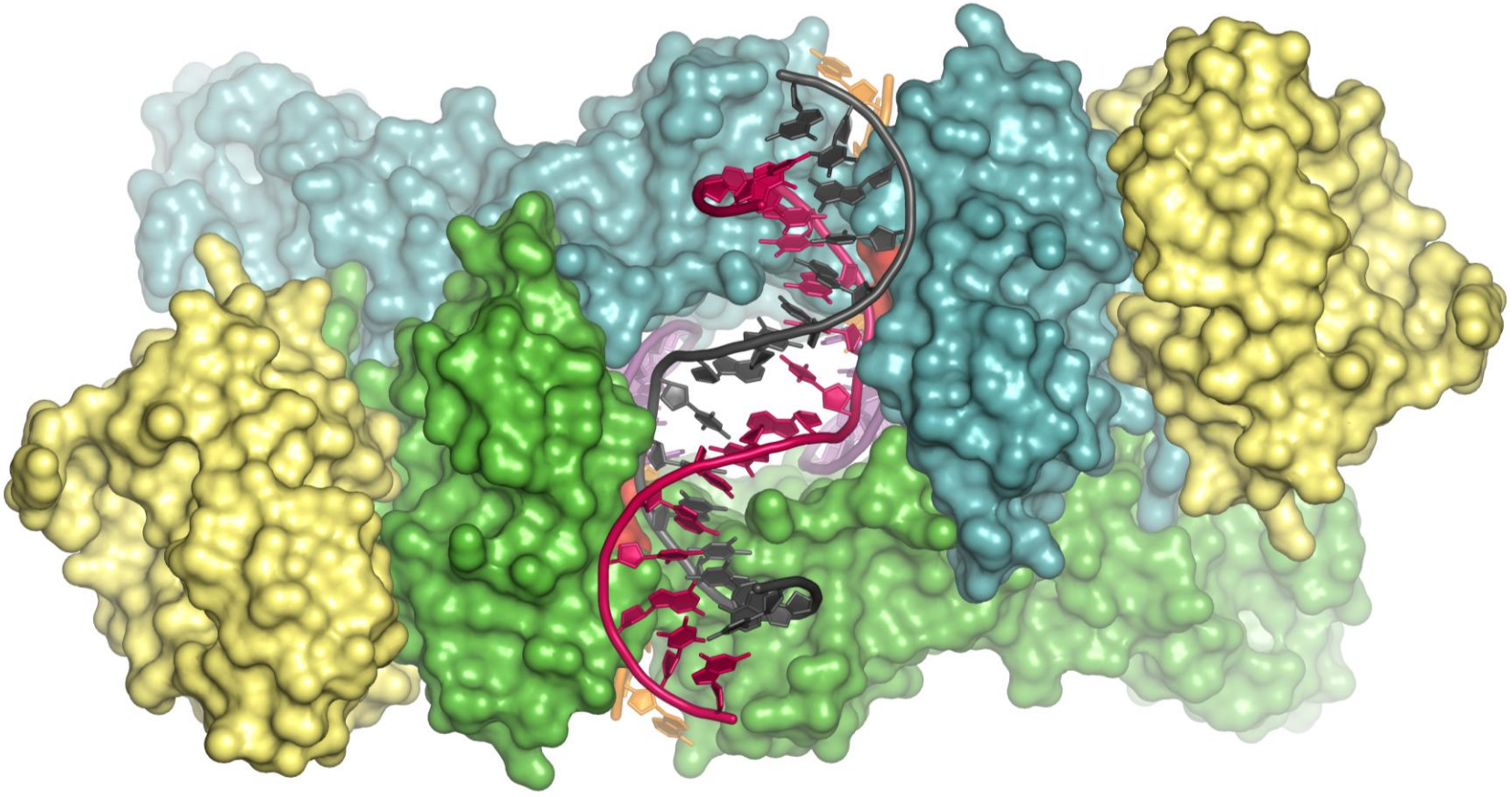
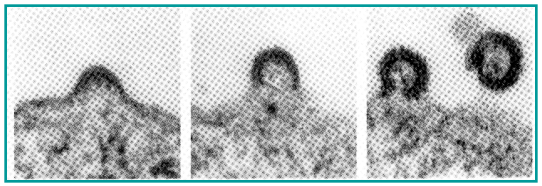
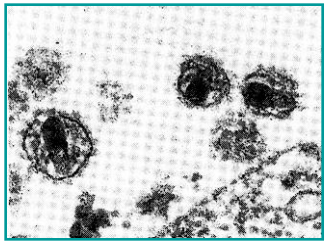
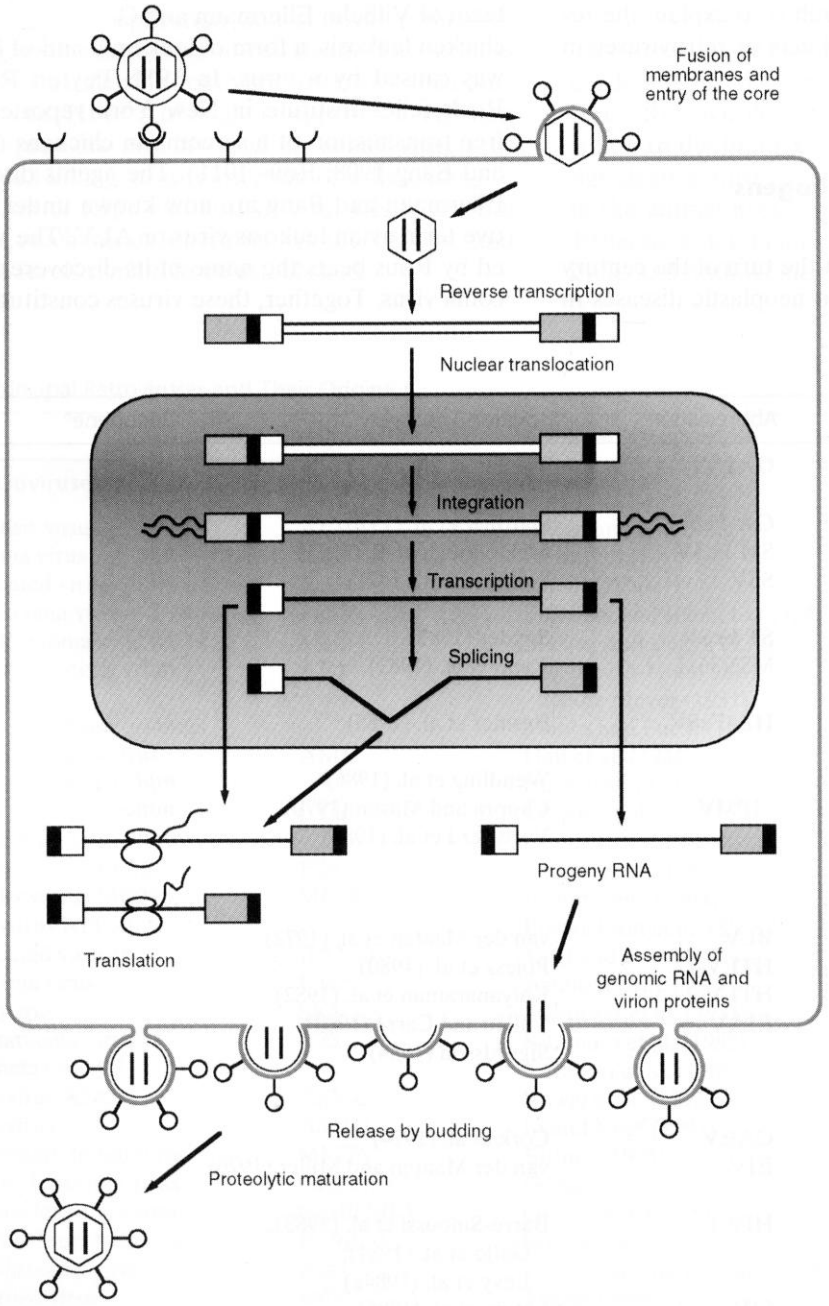
Retroviral DNA integration








Fusion of membranes and entry of the core
Reverse transcription
Nuclear translocation
Integration
Transcription
Splicing
Progeny RNA
Translation
Assembly of genomic RNA and virion proteins
Release by budding
Proteolytic maturation

Replication steps: Drug targets

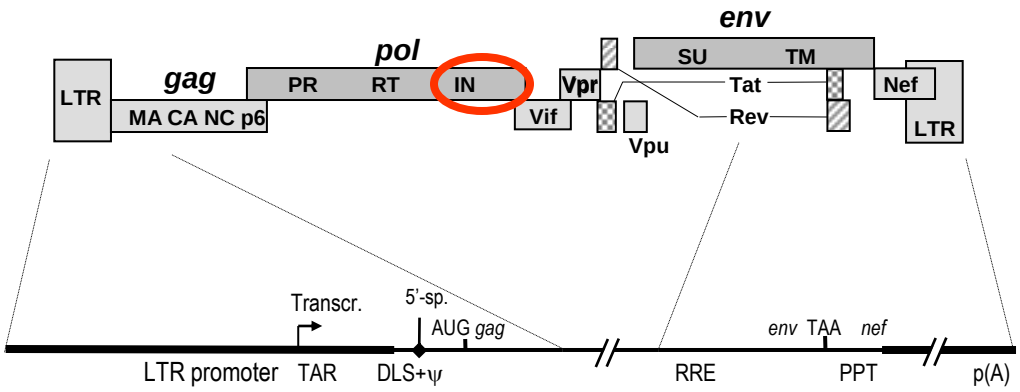
1) Binding to CD4
2) ~~Engagement of co-recepter and entry by fusion~~
3) ~~Reverse transcription~~
4) Nuclear import and integration into chromosome
5) Transcription from HIV LTR promoter
6) Assembly of new virions
7) Budding
8) ~~Protease-dependent maturation~~

Clinically approved inhibitors:

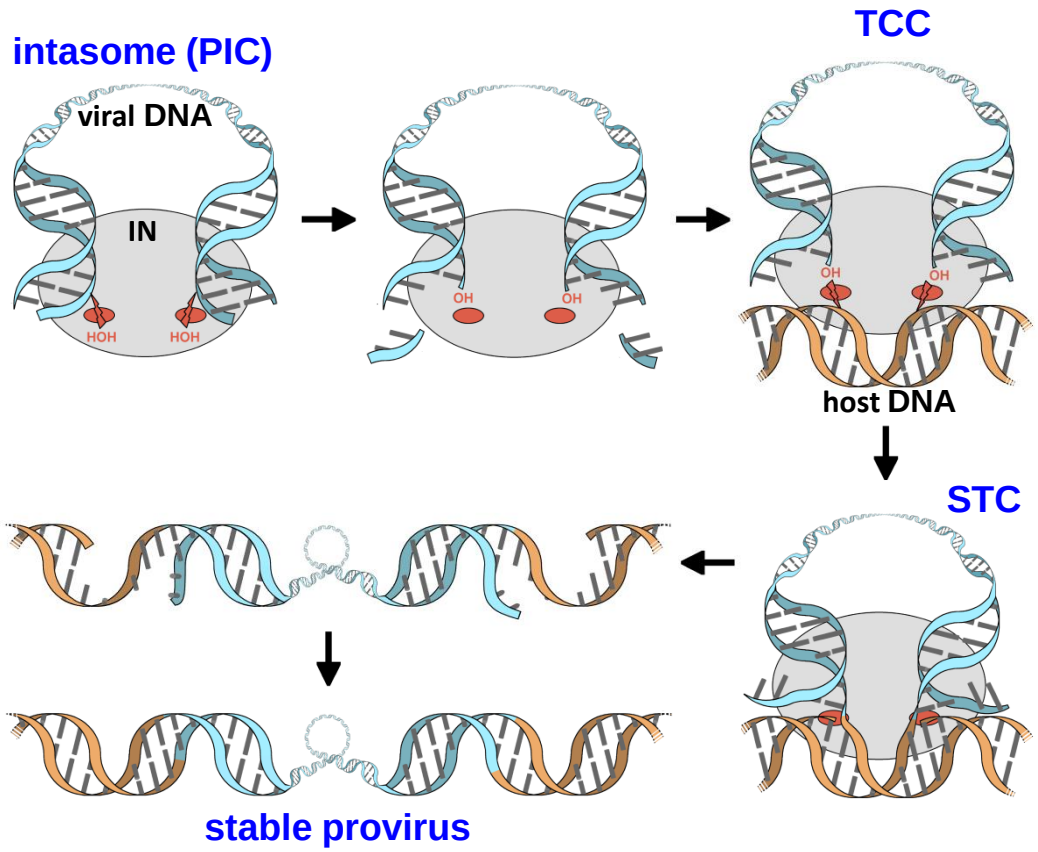
Brand Name	Generic Name	Manufacturer	FDA
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)			
Combivir	lamivudine and zidovudine	GlaxoSmithKline	1997
Emtriva	emtricitabine, FTC	Gilead Sciences	2003
Epivir	lamivudine, 3TC	GlaxoSmithKline	1995
Epzicom	abacavir and lamivudine	GlaxoSmithKline	2004
Hivid	zalcitabine, dideoxycytidine, ddC	Hoffmann-La Roche	1992
Retrovir	zidovudine, azidothymidine, AZT, ZDV	GlaxoSmithKline	1987
Trizivir	abacavir, zidovudine, and lamivudine	GlaxoSmithKline	2000
Truvada	TDF and emtricitabine	Gilead Sciences, Inc.	2004
Videx EC	enteric coated didanosine, ddI EC	Bristol Myers-Squibb	2000
Videx	didanosine, dideoxyinosine, ddI	Bristol Myers-Squibb	1991
Viread	tenofovir disoproxil fumarate, TDF	Gilead	2001
Zerit	stavudine, d4T	Bristol Myers-Squibb	1994
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)			
Ziagen	abacavir sulfate, ABC	GlaxoSmithKline	1998
Intelence	etravirine	Tibotec Therapeutics	2008
Rescriptor	delavirdine, DLV	Pfizer	1997
Sustiva	efavirenz, EFV	Bristol Myers-Squibb	1998
Viramune	nevirapine, NVP	Boehringer Ingelheim	1996
Protease Inhibitors (PIs)			
Agenerase	amprenavir, APV	GlaxoSmithKline	1999
Aptivus	tipranavir, TPV	Boehringer Ingelheim	2005
Crixivan	indinavir, IDV,	Merck	1996
Fortovase	saquinavir (no longer marketed)	Hoffmann-La Roche	1997
Invirase	saquinavir mesylate, SQV	Hoffmann-La Roche	1995
Fusion and Entry Inhibitors			
Fuzeon	enfuvirtide, T-20	Hoffmann-La Roche & Trimeris	2003
Selzentry	maraviroc	Pfizer	2007
HIV integrase strand transfer inhibitors (InSTIs)			
Isentress	Raltegravir, MK0518	Merck	2007

Genomic organization of HIV

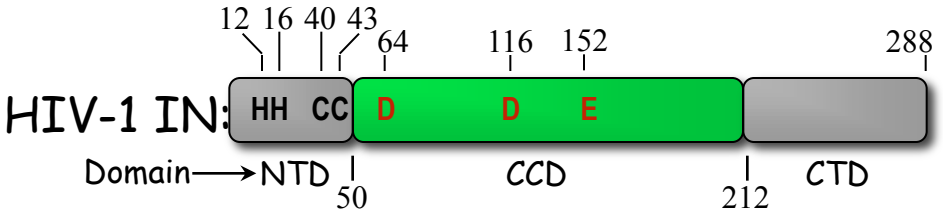
(proviral form)



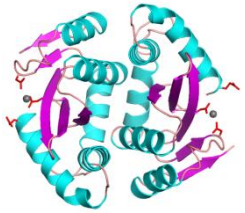
Catalytic functions of retroviral integrase



IN "shotgun crystallography": early work

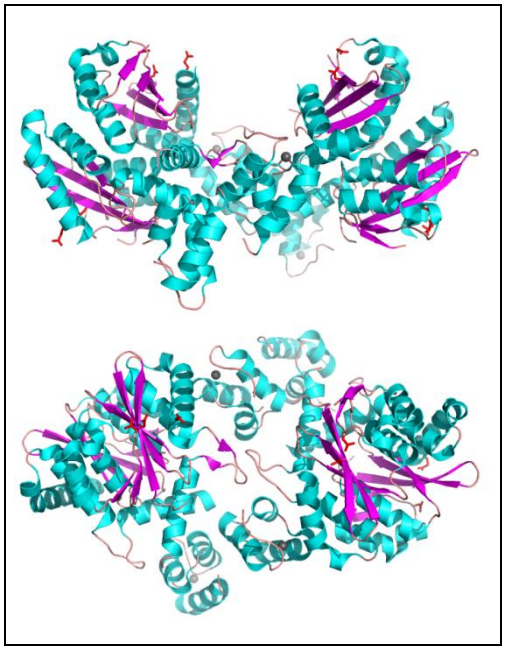


Cai *et al.*, NSMB, 1997

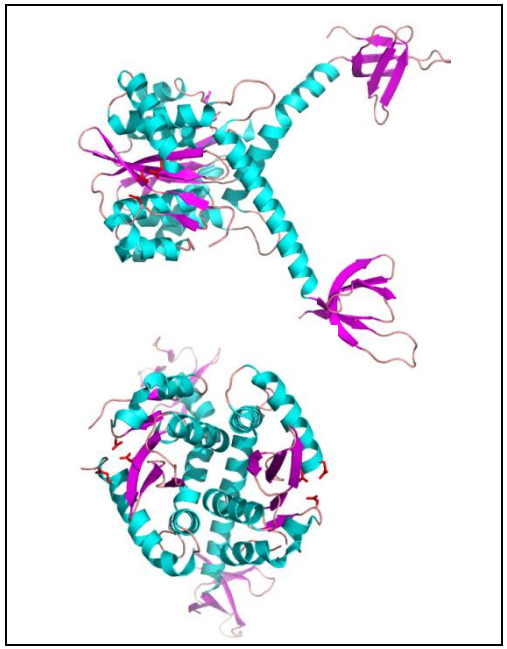


Dyda *et al.*, Science 1994

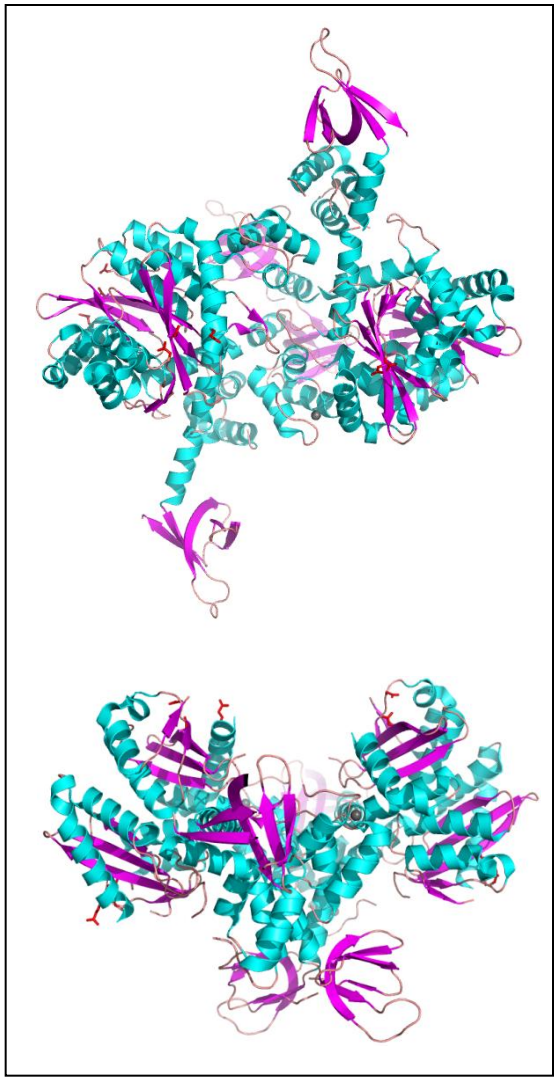
Eijkelenboom *al.*, NSMB, 1995



Wang *et al.*, EMBO J., 2001

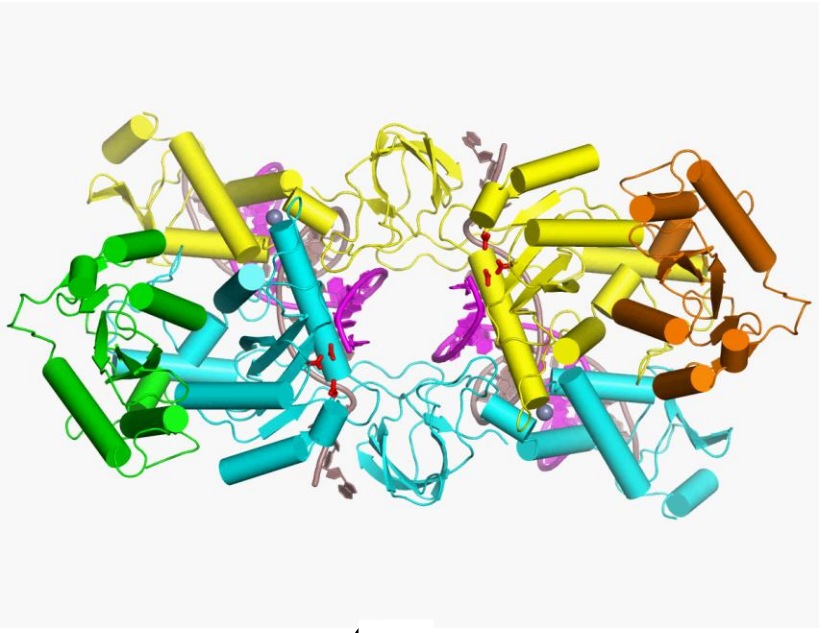


Chen *et al.*, PNAS, 2000

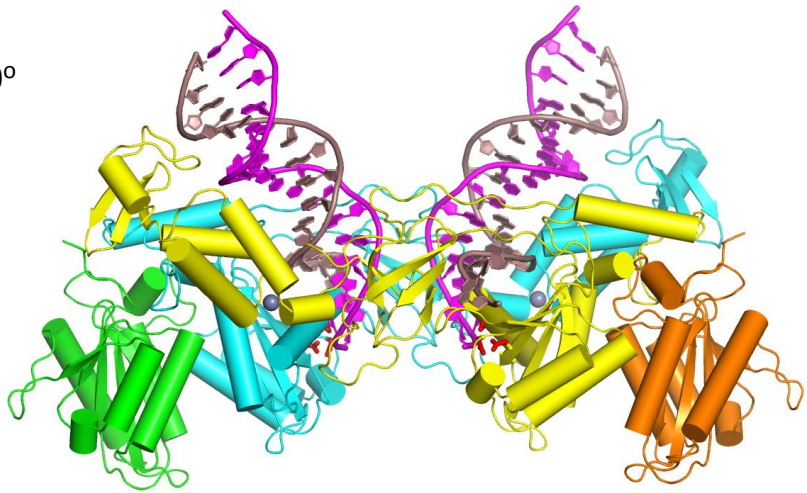


Composite models

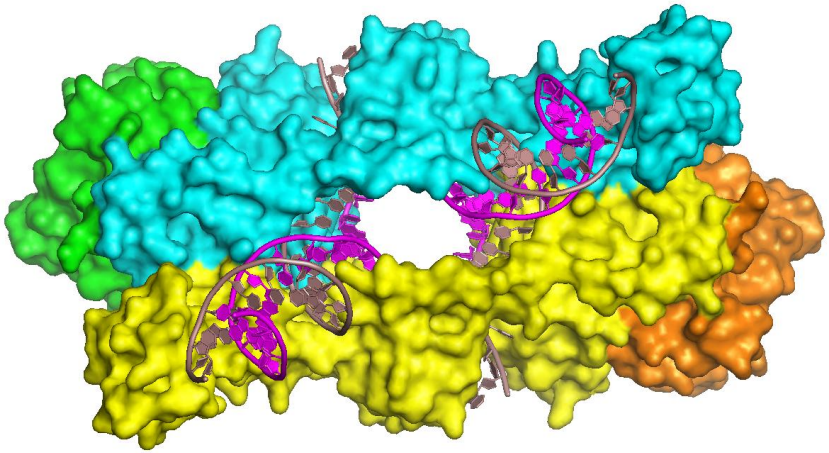
Overall architecture of the pre-integration complex (intasome)



90°



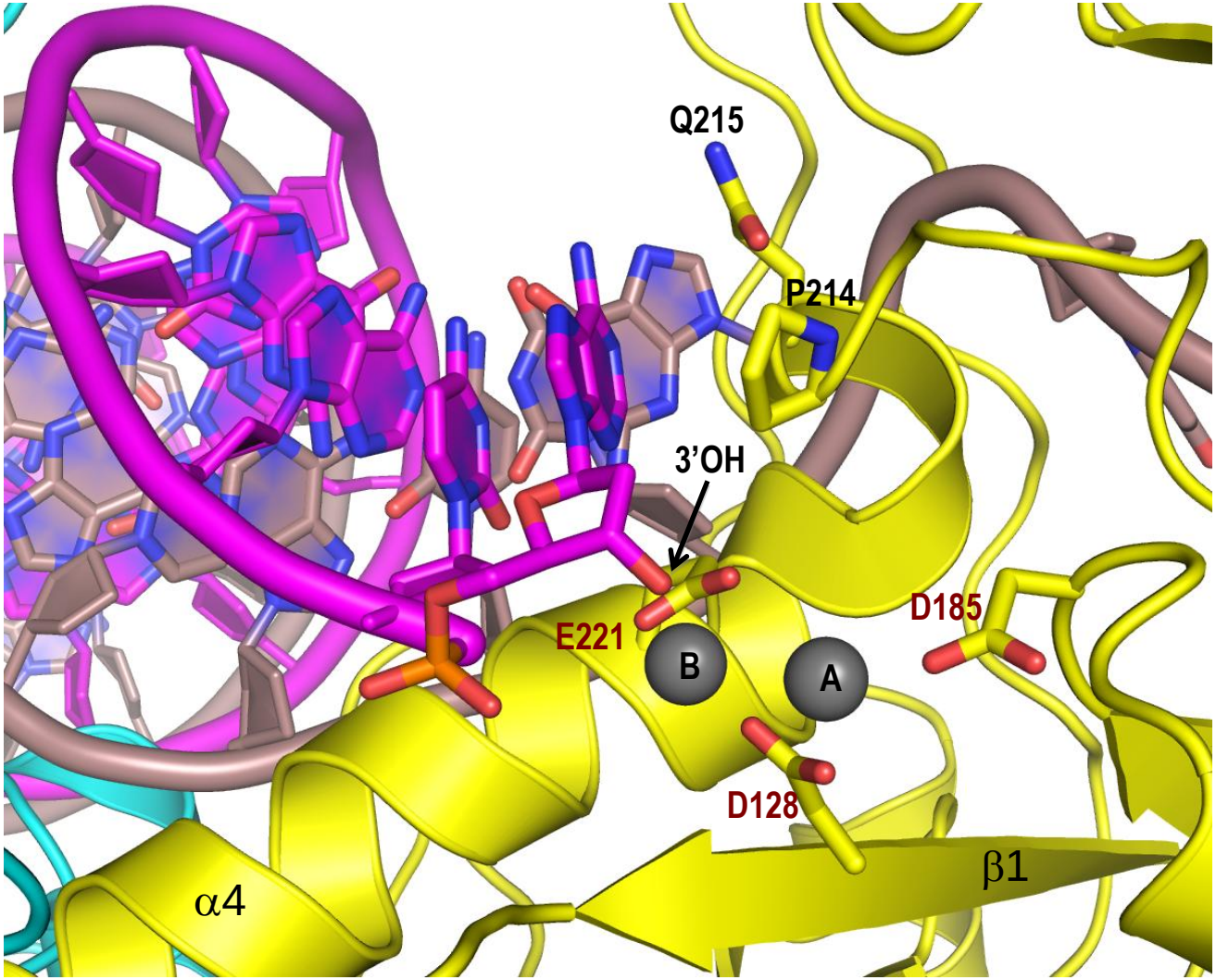
180°



IN tetramer:
dimer of dimers, ~26 Å active site separation

Hare *et al.*, Nature, 2010

Active site (prior to binding target DNA)



Hare S. et al, Nature, 2010

Retroviral integrase family

"Relatives" of retroviral integrases:

(based on structural organization and active site mechanics)

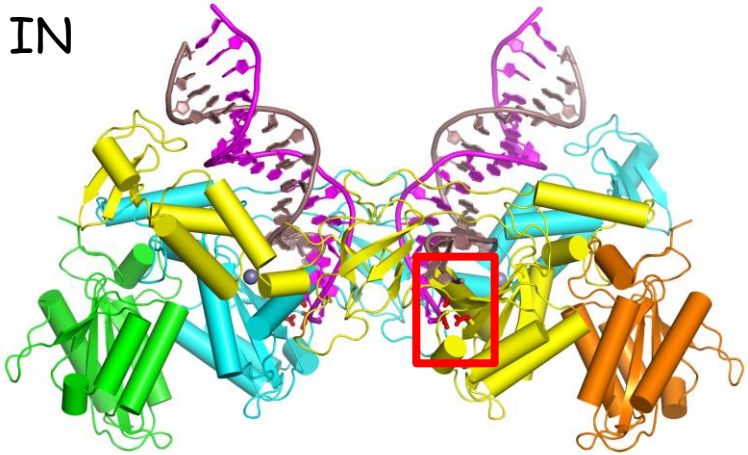
Integrases from retrotransposons (yeast Ty5, Ty3, *etc*)
Eukaryotic transposases (Tc1, Sleeping Beauty)
Prokaryotic transposases
    conservative (cut-and-paste) transposition: Tn5, Tn10, Tn7
    replicative transposition: Tn3, Mu phage, *etc*.
Holliday junction resolvases (RuvC)
V(D)J recombinase RAG1/2
RNaseH enzymes (HIV, *E. coli*, *etc*.)
Ago (=catalytic subunit of RNA-induced silencing complex [RISC])

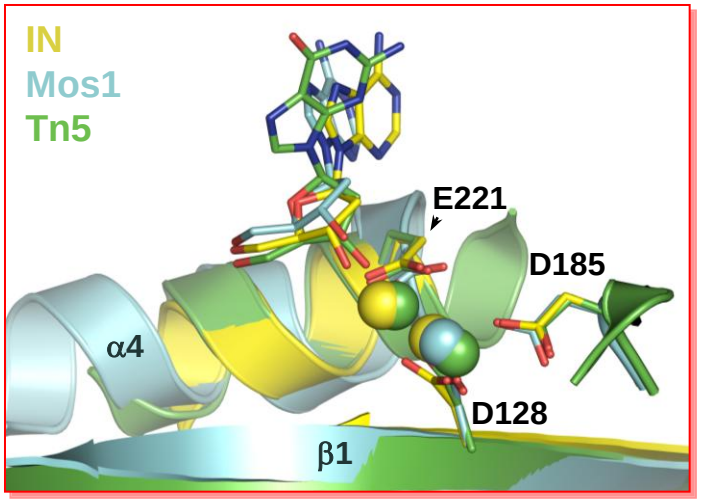
Int family

Retroviral integrases are **NOT** related to tyrosine recombinases
Such as bacteriophage lambda *integrase*, P1 recombinase Cre, yeast Flp recombinase, *etc.*

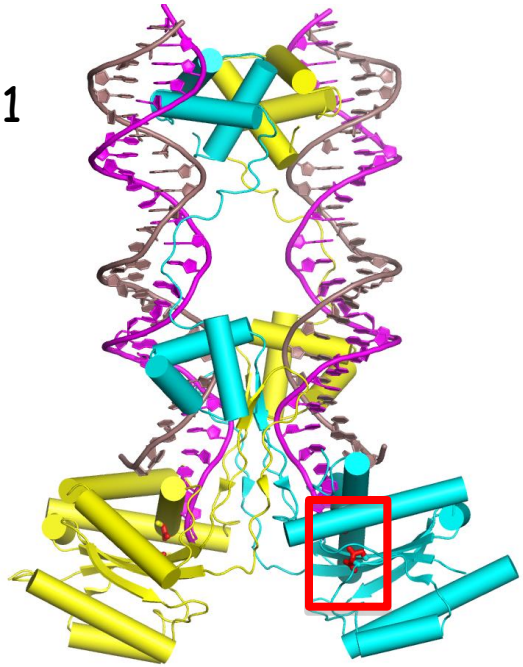
Comparison with another DDE(D) family members

IN

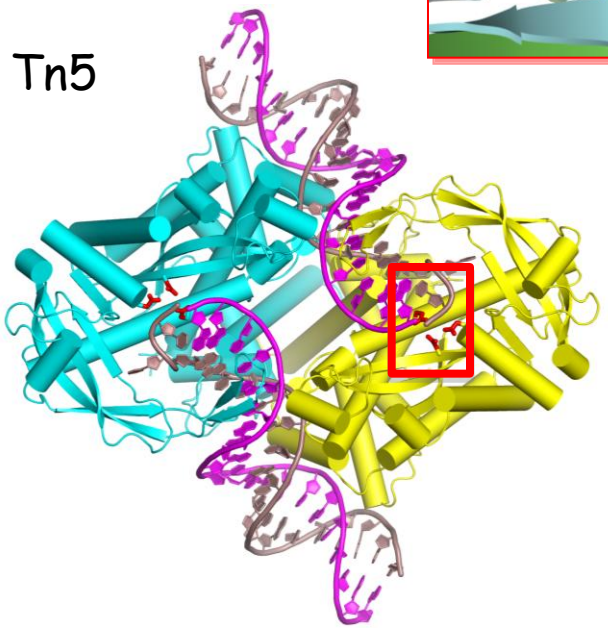




Mos1



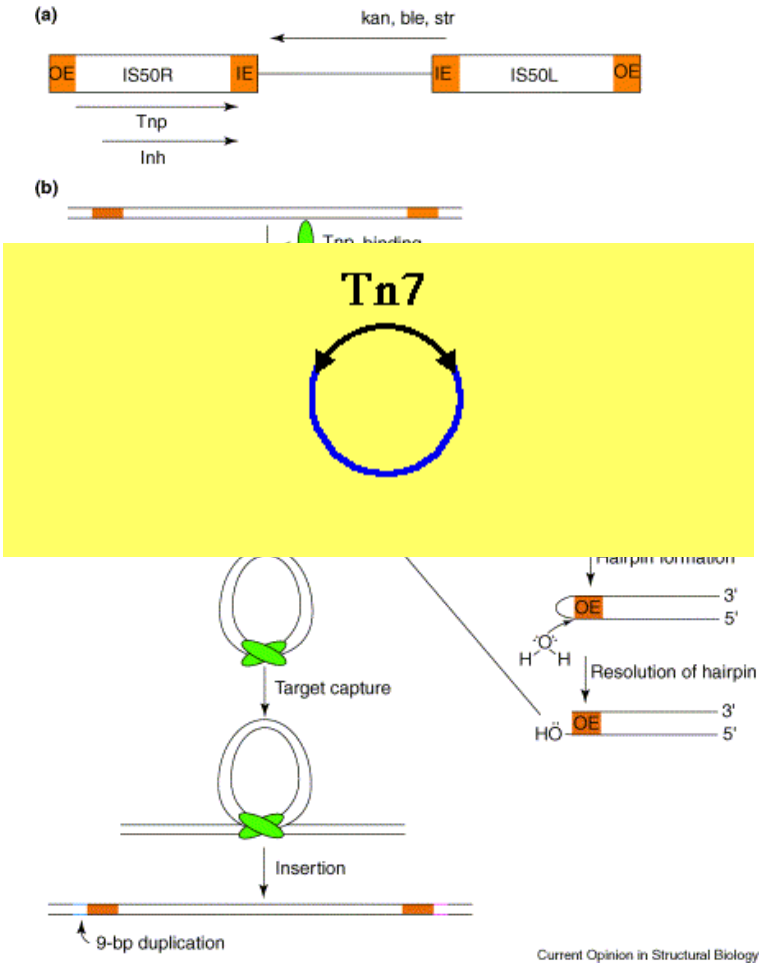
Tn5

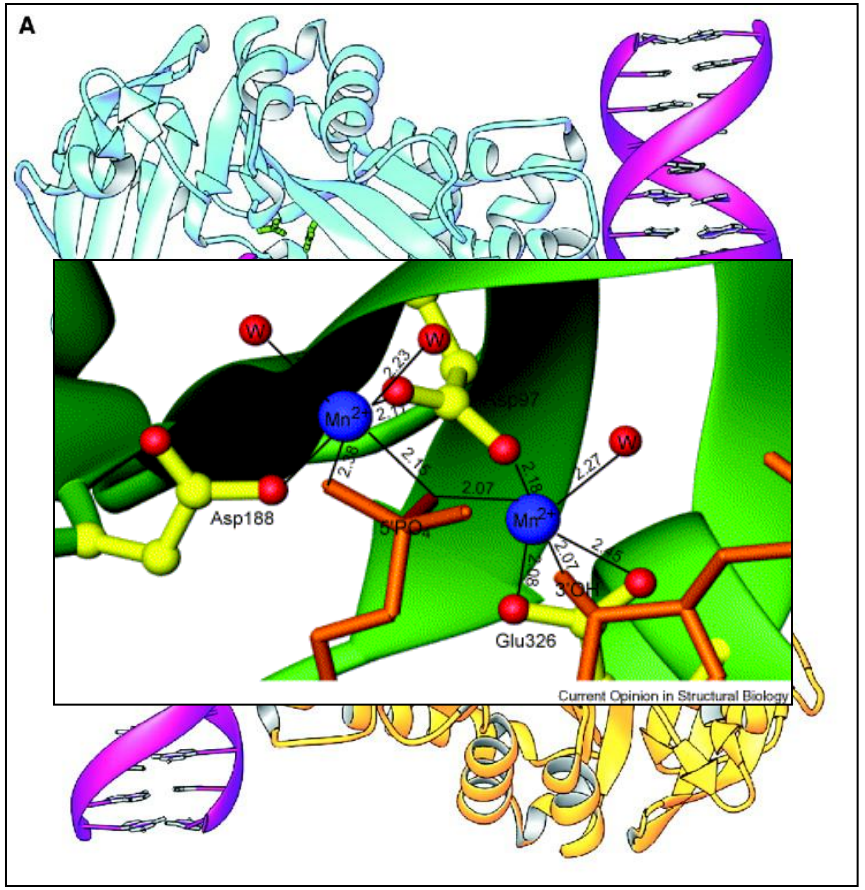


Richardson *et al.*, Cell, 2009, 138(6):1096-1108

Davis *et al.*, Science, 2000, 289(5476):77-85
Steiniger-White *et al.*, Curr. Opin. Struct. Biol., 2004, 14(1):50-57

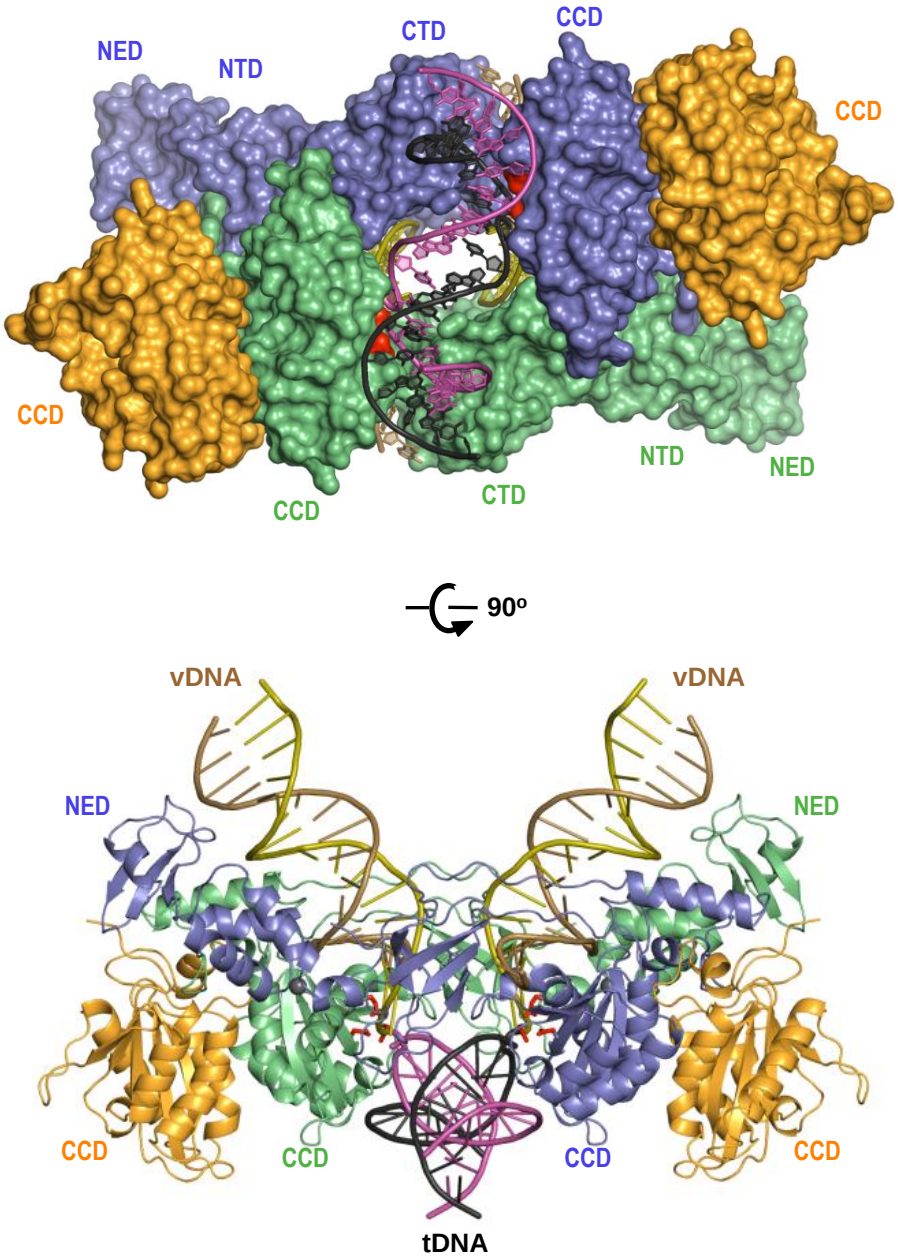
Prokaryotic transposases (Tn7, Tn5)





Steiniger-White M. et al, Current Opinion Struct Biol, 2004

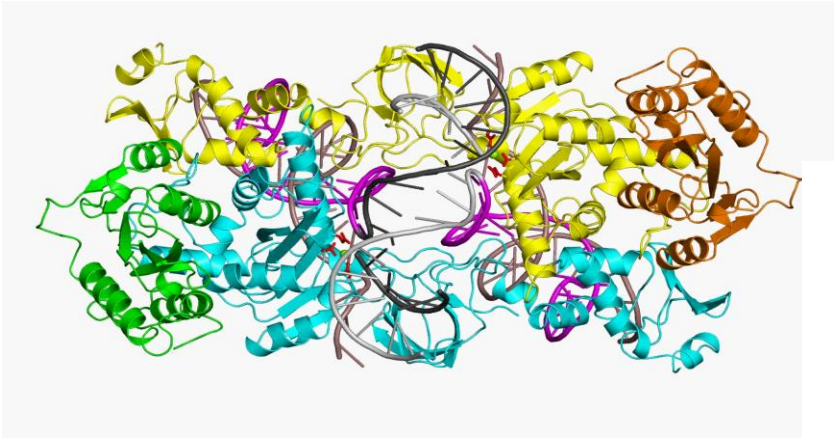
Strand transfer complex

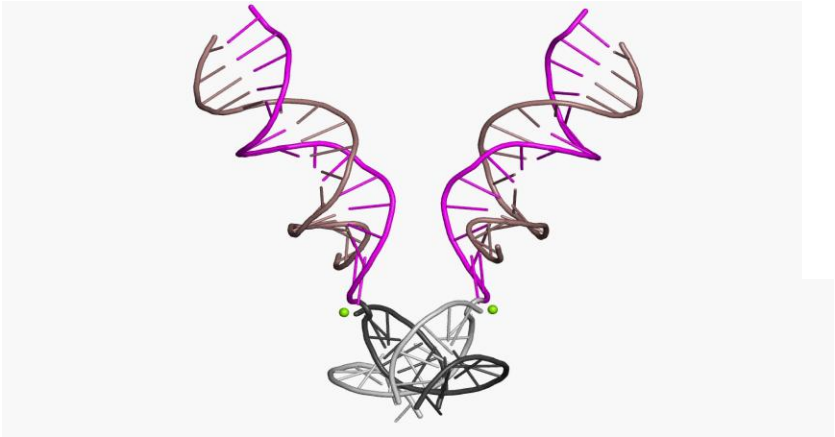


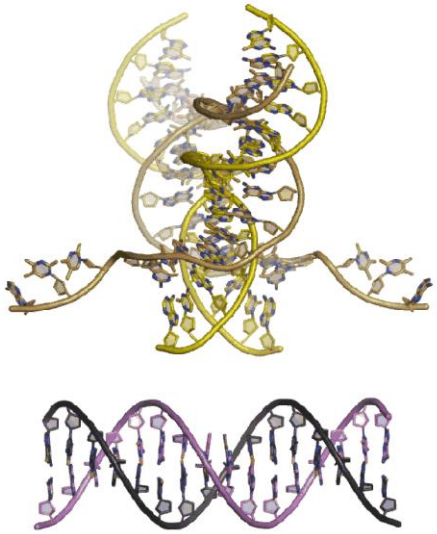
Maertens G. et al, Nature, 2010

Active site mechanics during strand transfer

Structure of the pre-integration complex bound to target DNA:



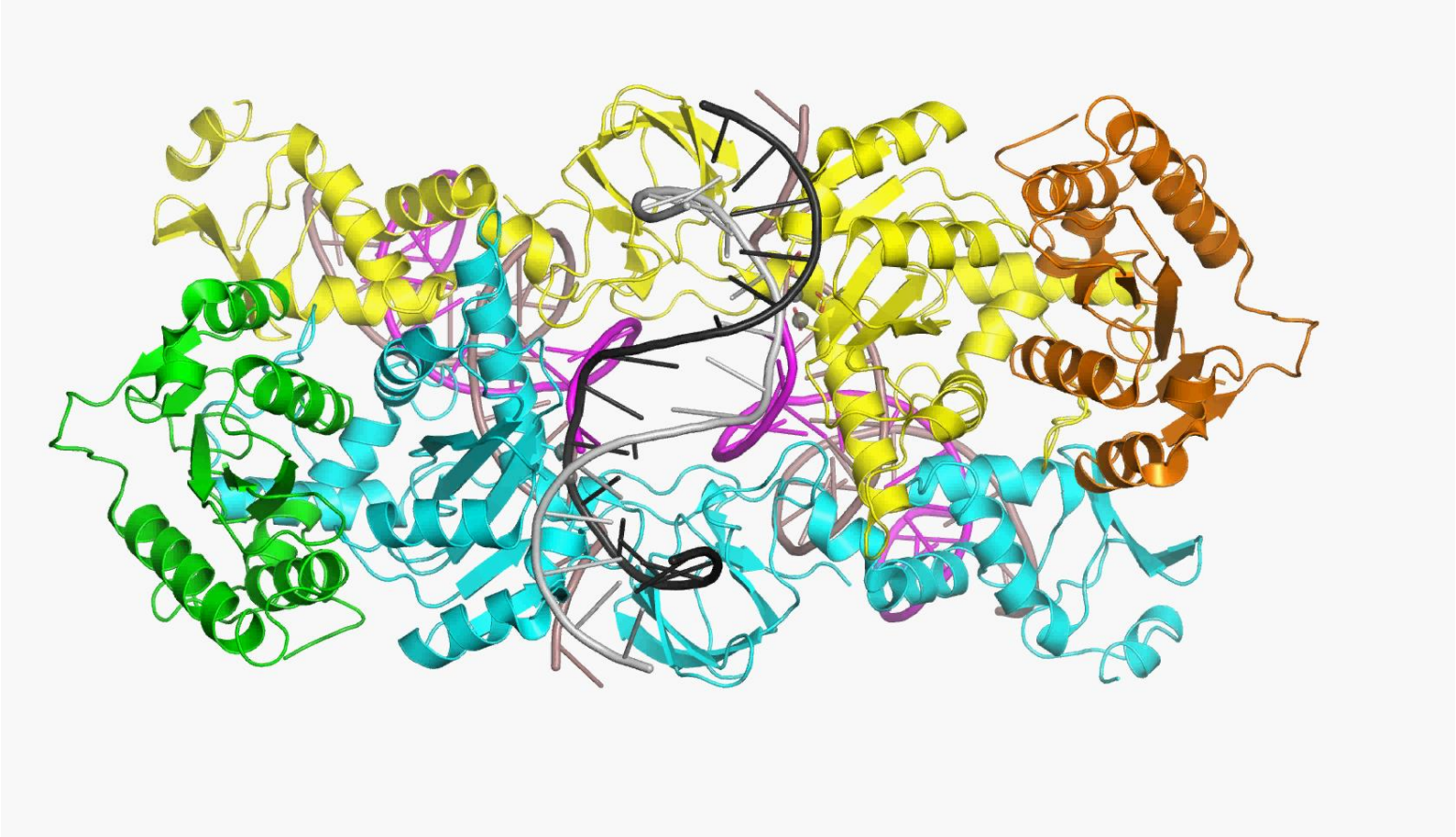




Maertens G. et al, Nature, 2010

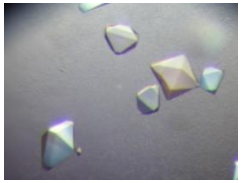
Active site mechanics during strand transfer

Model of metal-loaded TCC (superposition of 3L2S and TCC):

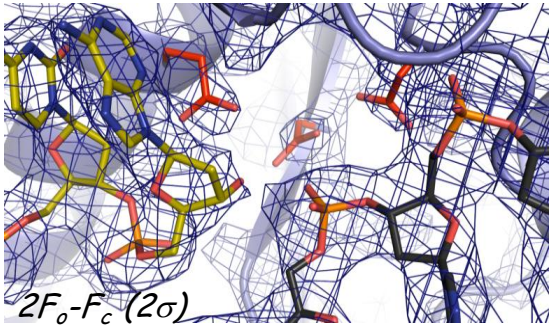


Strand transfer *in crystallo*

TCC_{EDTA}

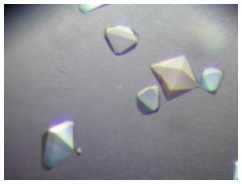


snap freeze



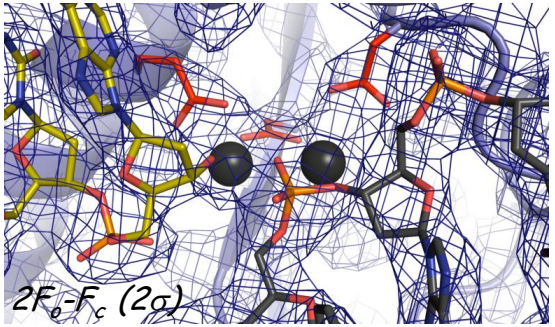
3.15 Å

TCC_{EDTA}



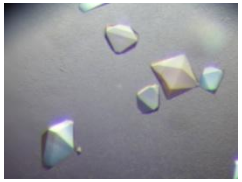
Soak in Mn^{2+}
~ 90 sec

snap freeze



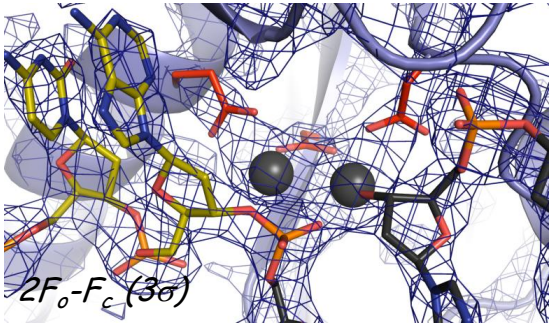
3.05 Å

TCC_{EDTA}



Soak in Mn^{2+}
~ 3 min

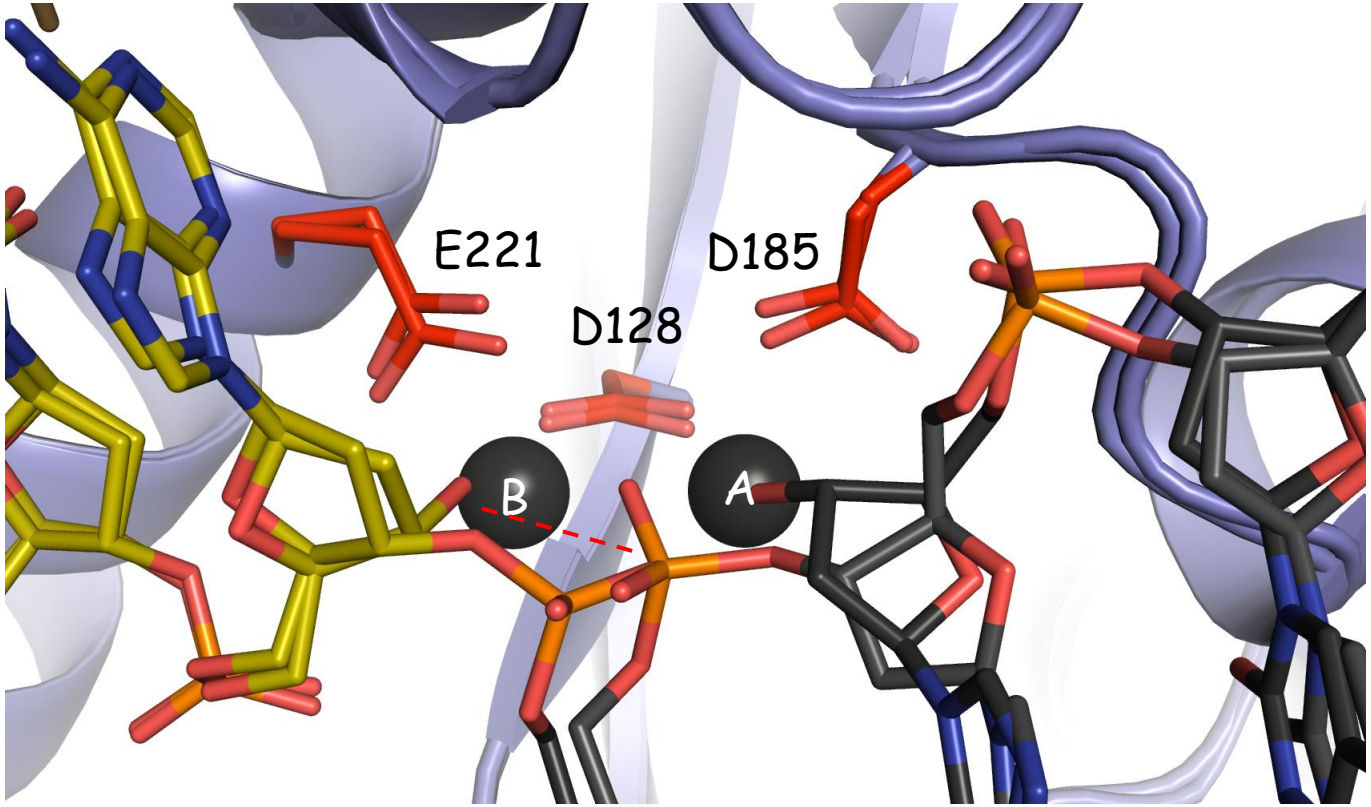
snap freeze



3.00 Å

Maertens G. et al, in preparation

Structural change in active site renders integration reaction irreversible



Maertens G. et al, in preparation

Clinical InSTIs:

MK-0518 (FDA approved) Merck

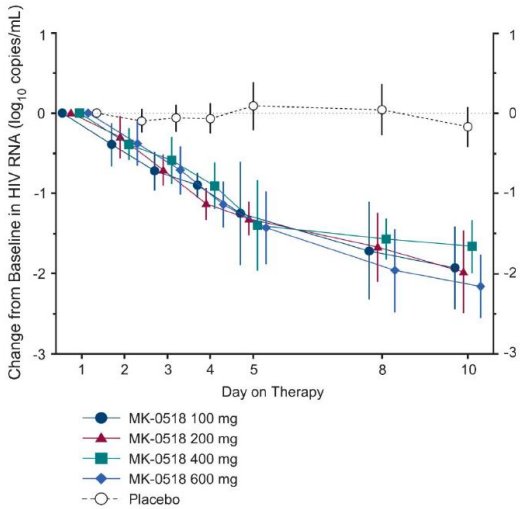
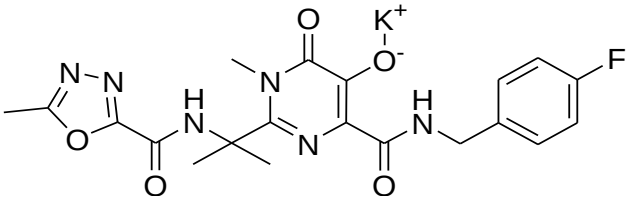


FIGURE 2. Change from baseline at day 10 in $\log_{10}$ HIV RNA (with 95% CI). Error bars indicate 95% CIs. Data for a total of 7 patients (from 4 of the 5 treatment groups) were not available on day 5.



GS-9137 (phase III), Gilead

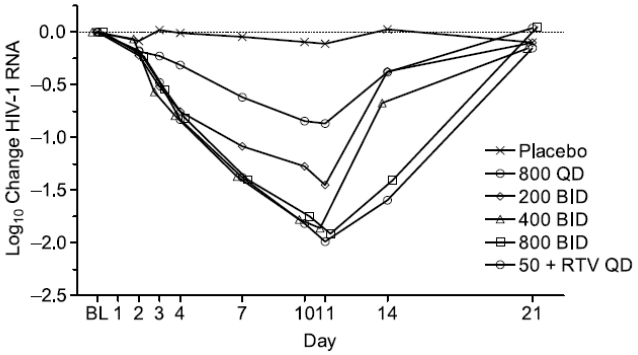
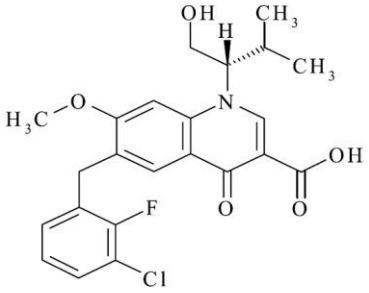
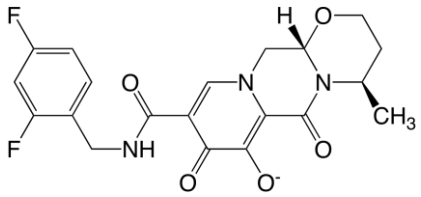


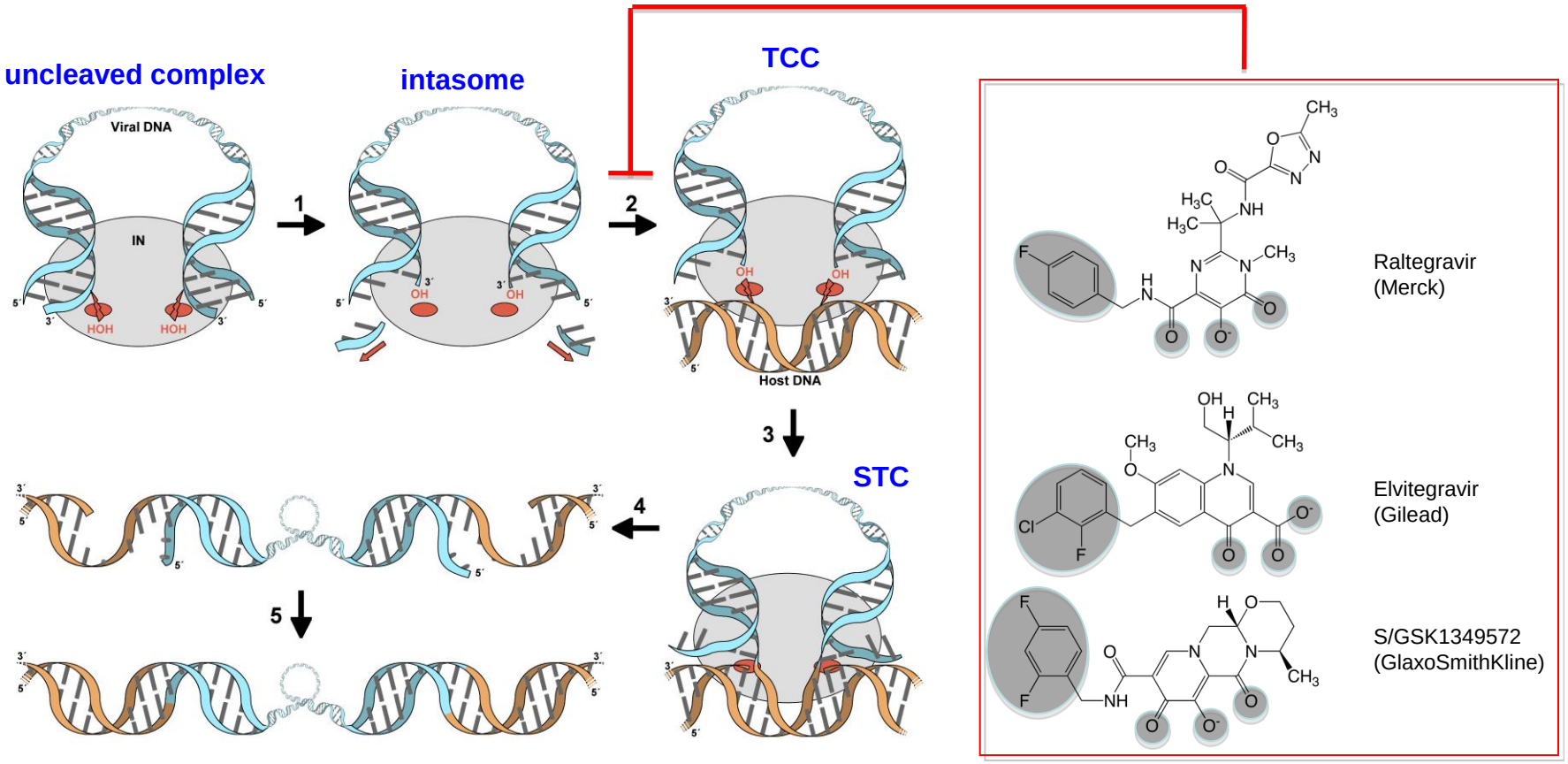
FIGURE 2. Change in HIV RNA from baseline over time.



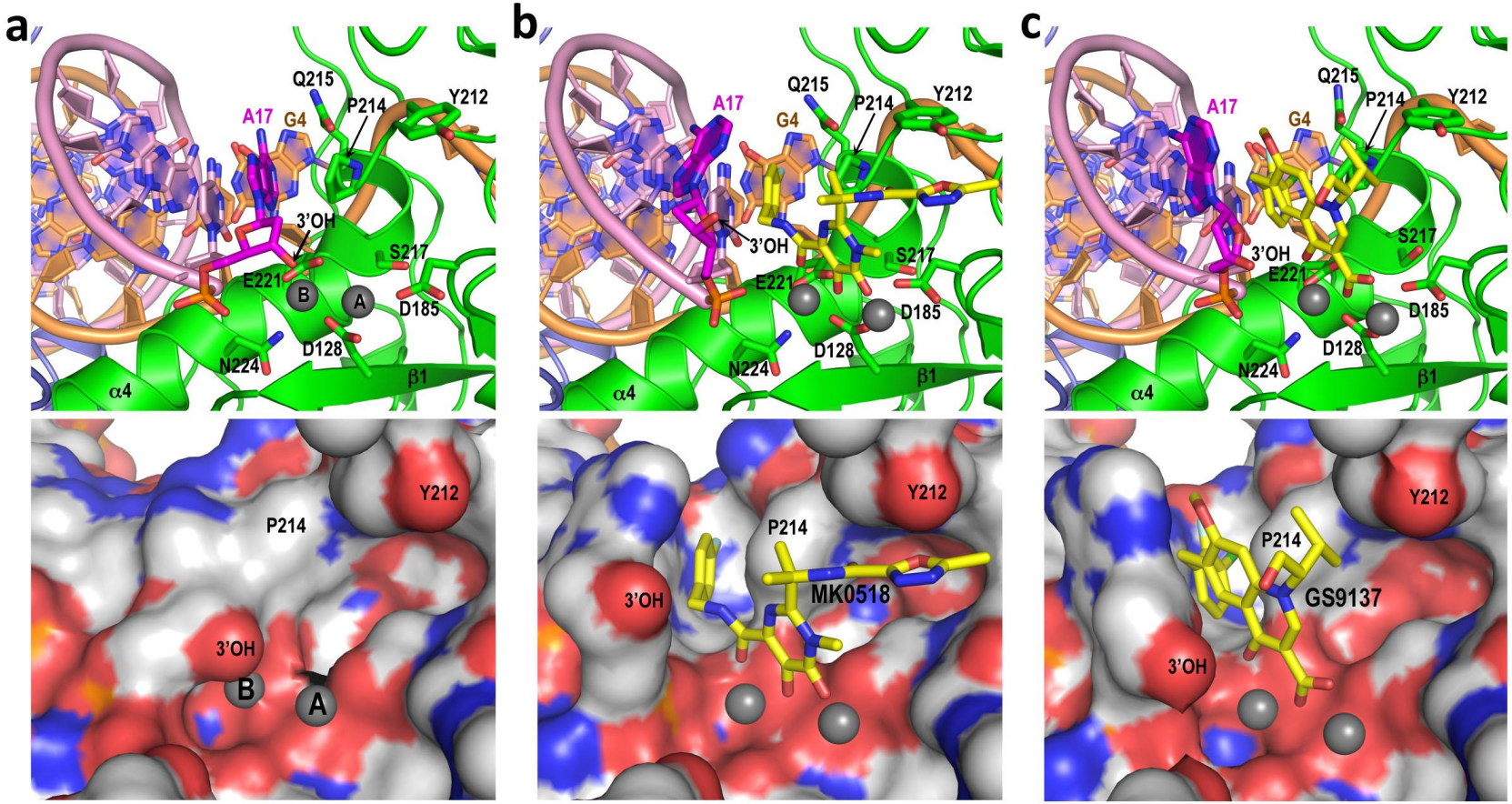
GSK-572 (phase II) Glaxo



Retroviral integration

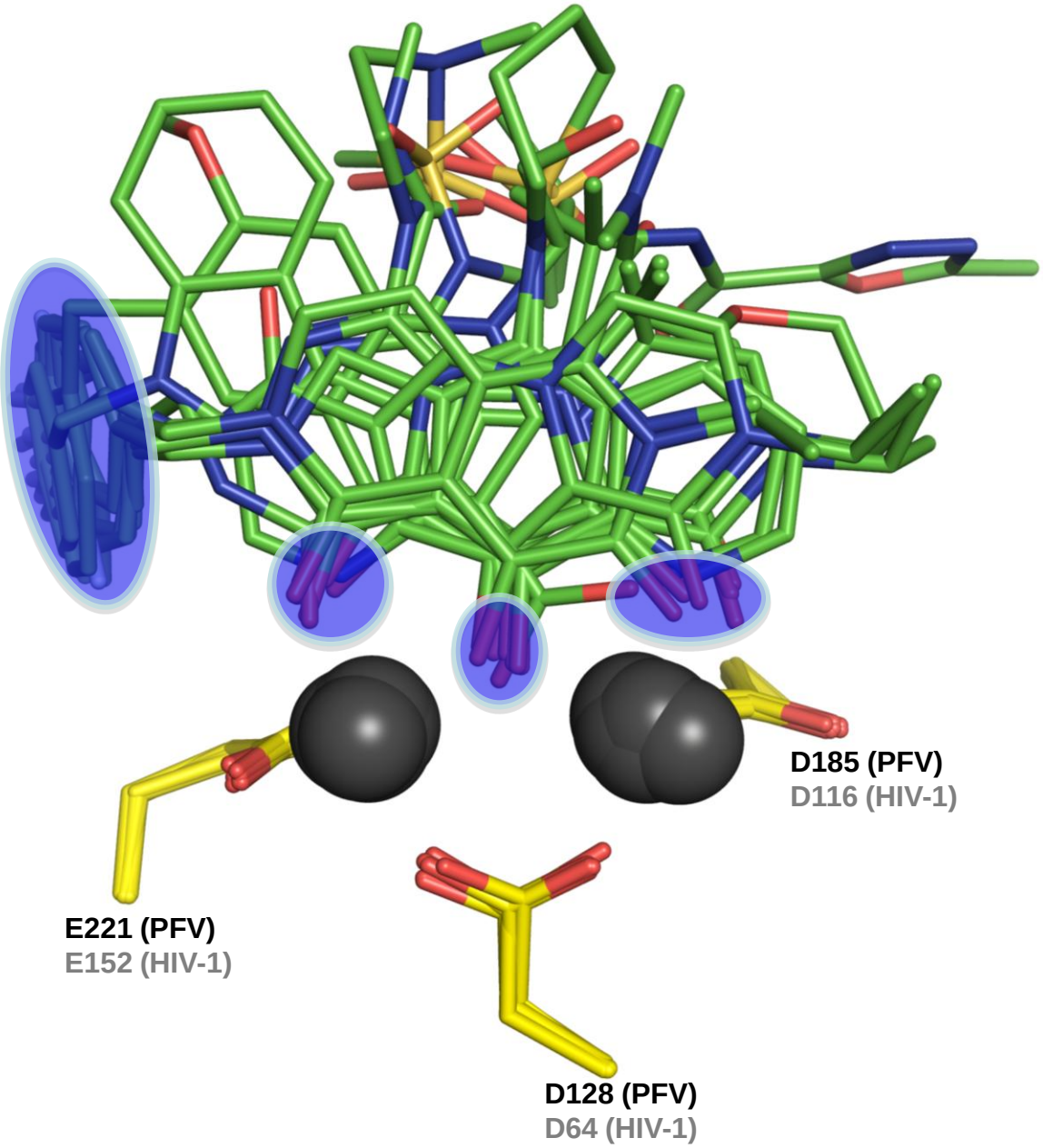


Mechanism of InSTI action



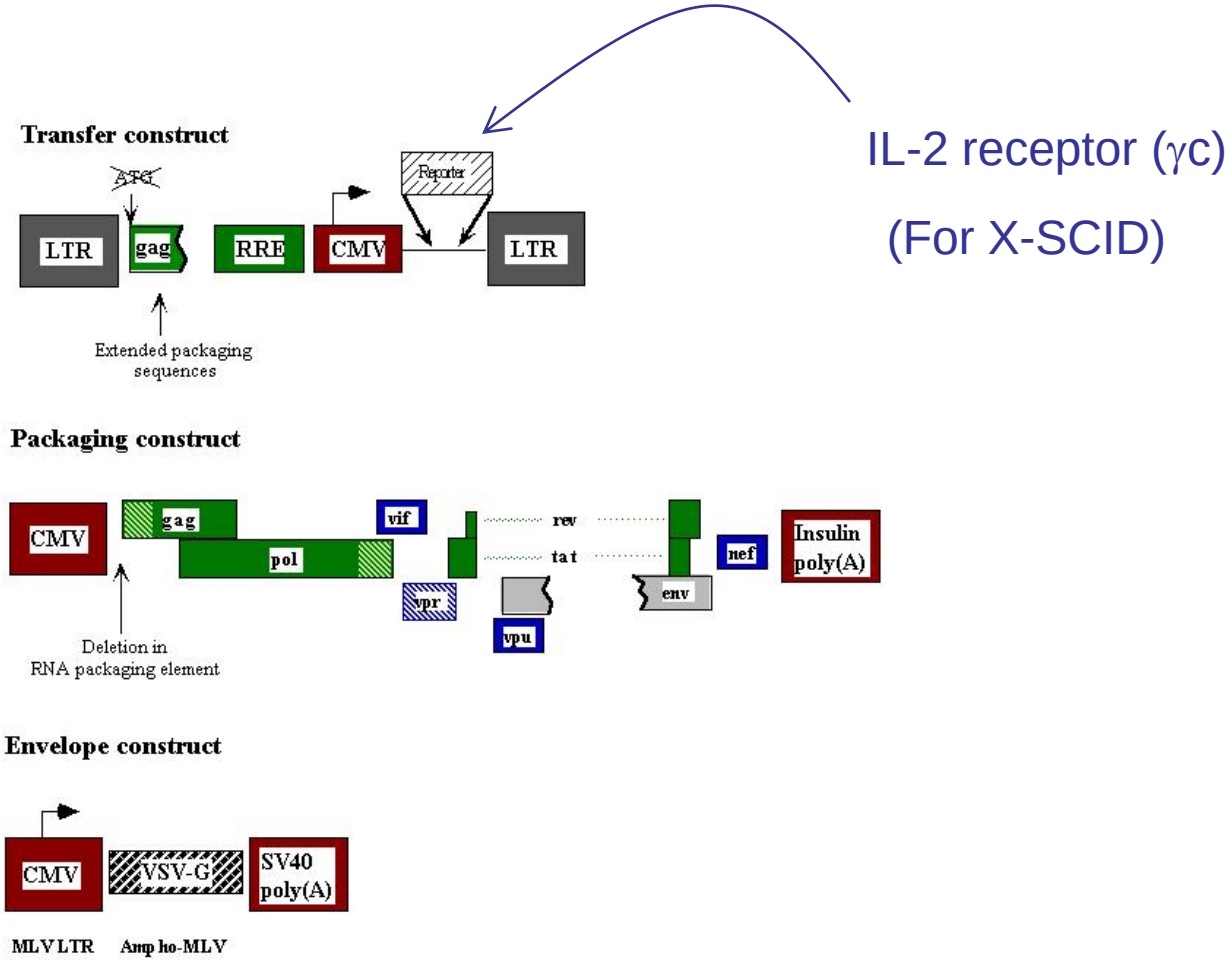
Hare et al., Nature, 2010

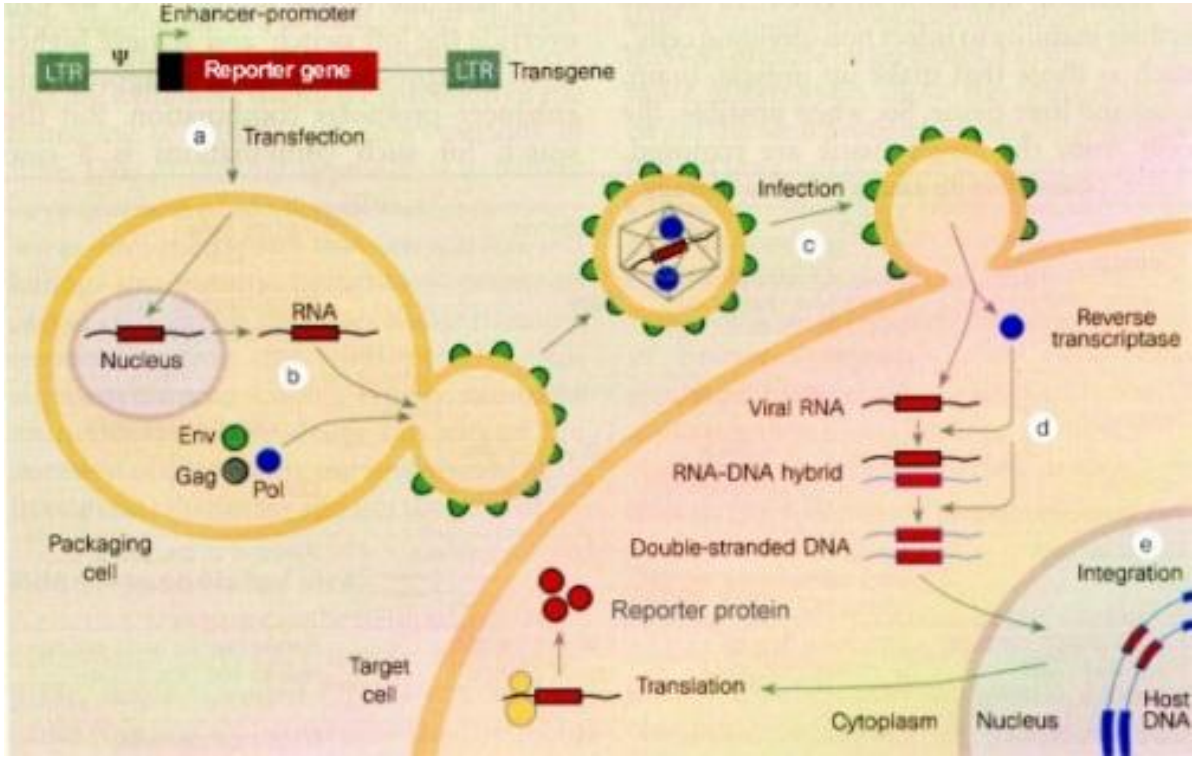
Consensus INSTI pharmacophore



Hare et al., PNAS, 2010

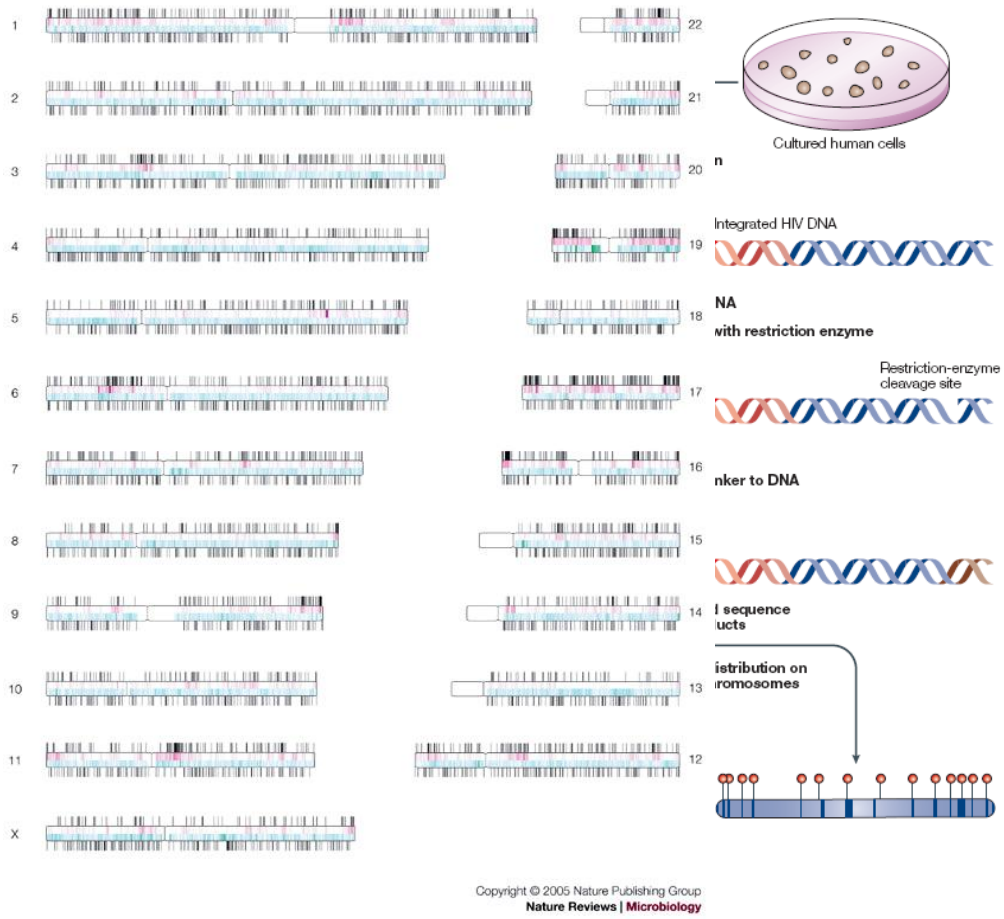
Applications to gene therapy


Transfer construct
ΔPsi
LTR
gag
RRE
CMV
Reporter
LTR
Extended packaging sequences
Packaging construct
CMV
gag
pol
vif
vpr
vpu
rev
tat
env
nef
Insulin poly(A)
Deletion in RNA packaging element
Envelope construct
CMV
VSV-G
SV40 poly(A)
MLV LTR
Ampho-MLV
IL-2 receptor (γc)
(For X-SCID)


Enhancer-promoter
LTR
Ψ
Reporter gene
LTR
Transgene
a
Transfection
Infection
c
Nucleus
RNA
b
Env
Gag
Pol
Packaging cell
Reverse transcriptase
Viral RNA
d
RNA-DNA hybrid
Double-stranded DNA
e
Integration
Reporter protein
Target cell
Translation
Cytoplasm
Nucleus
Host DNA

Do retroviruses favor certain chromosomal loci for integration ?

GENOME-WIDE ANALYSIS OF RETROVIRAL DNA INTEGRATION:

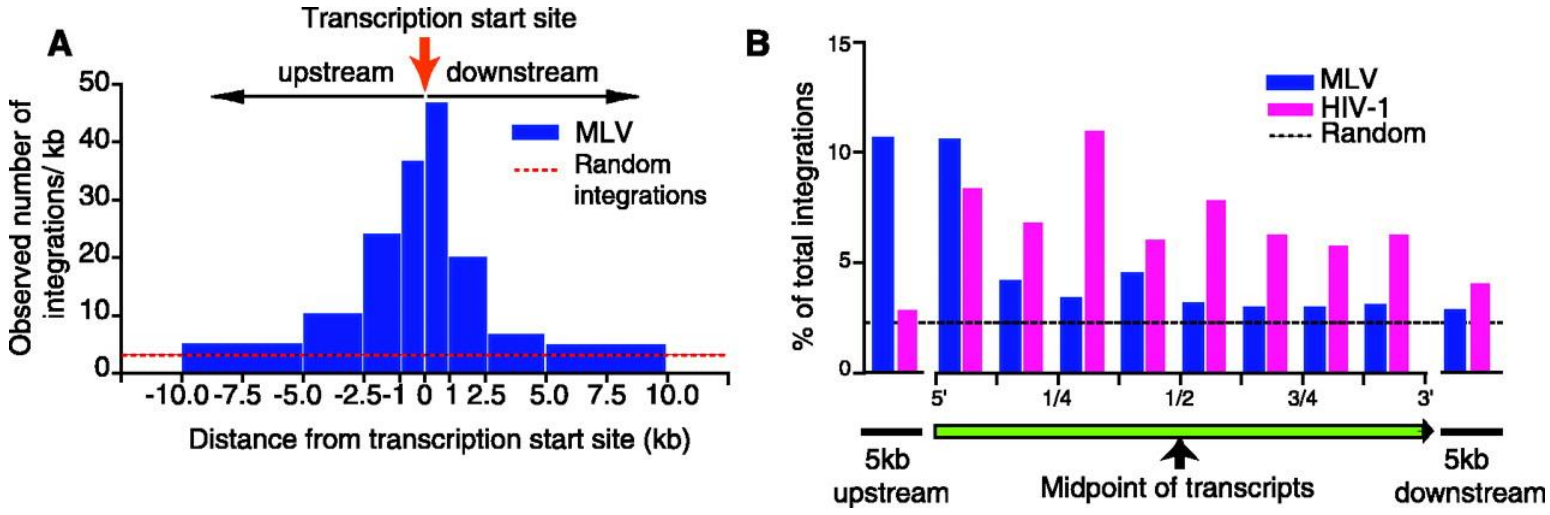


~73% (*v.s.* 31%)

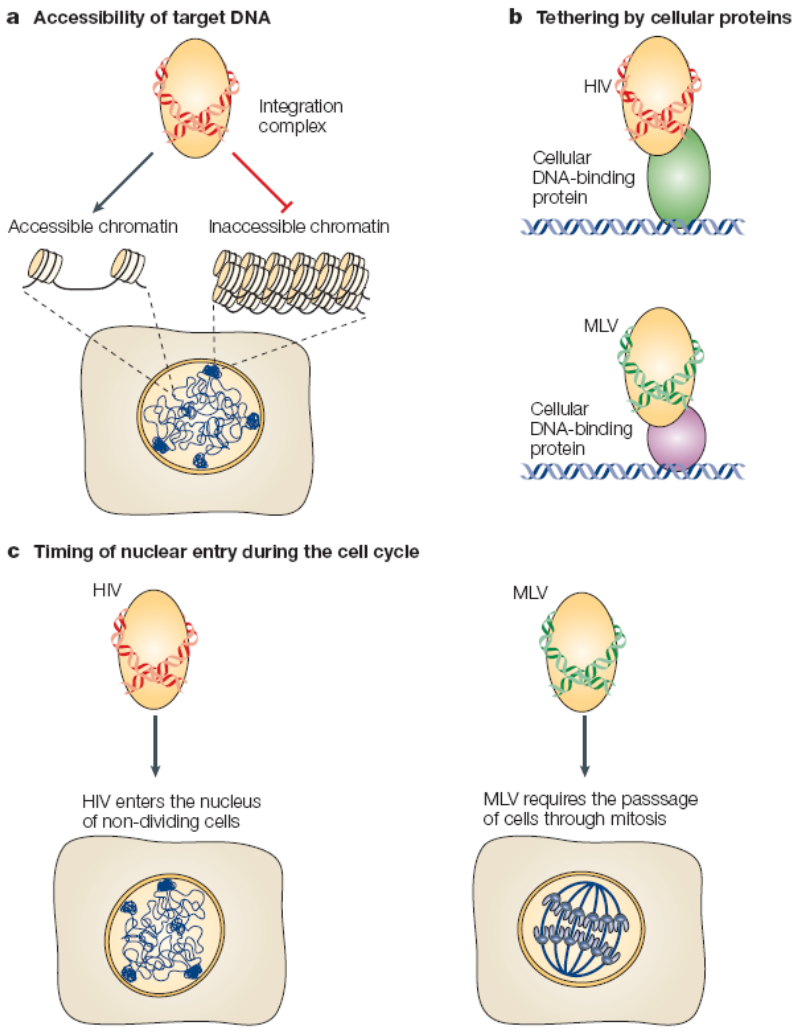
HIV-1 proviruses are in transcription units

~20% (*v.s.* 4%)
MLV proviruses are in promoter regions

Mitchell RS et al, PLoS Biol, 2004


A
Transcription start site
upstream
downstream
MLV
Random integrations
Observed number of integrations/ kb
50
40
30
20
10
0
-10.0 -7.5 -5.0 -2.5 -1 0 1 2.5 5.0 7.5 10.0
Distance from transcription start site (kb)
B
% of total integrations
15
10
5
0
MLV
HIV-1
Random
5'
1/4
1/2
3/4
3'
5kb upstream
Midpoint of transcripts
5kb downstream

How do we explain the differences between retroviruses?

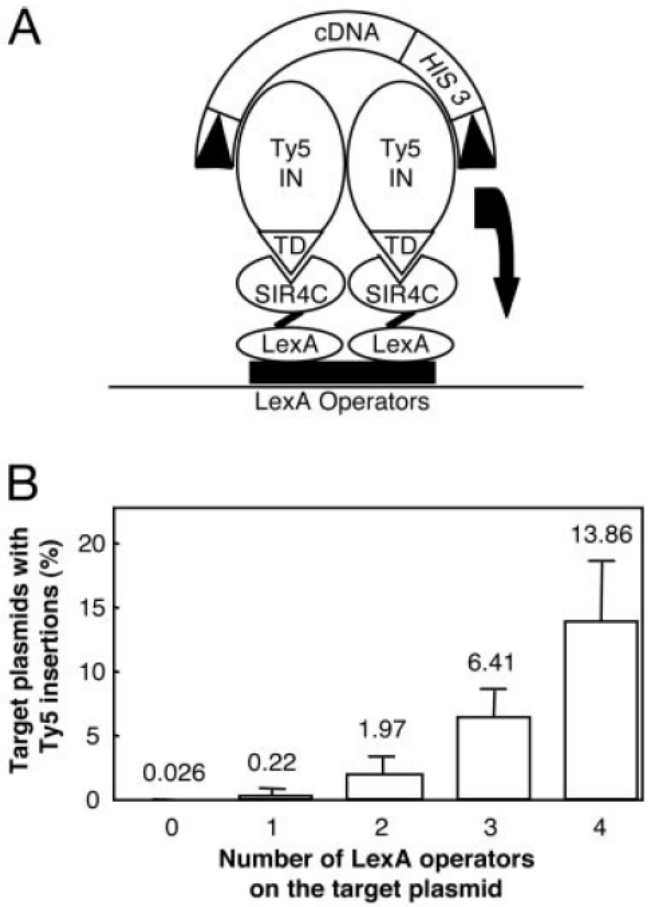


Target site selection by yeast retrotransposons

Ty5: transcriptionally-silent heterochromatin -> Sir4
Ty1, Ty3: RNA polymeraseIII (tRNA) genes -> TFIIIB

"tethering model"

Controlling Ty5 integration:



Zhu Y et al, PNAS, 2003

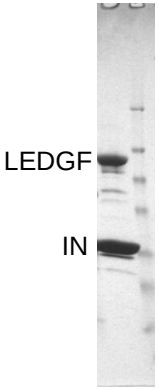
Any targeting factors for HIV?

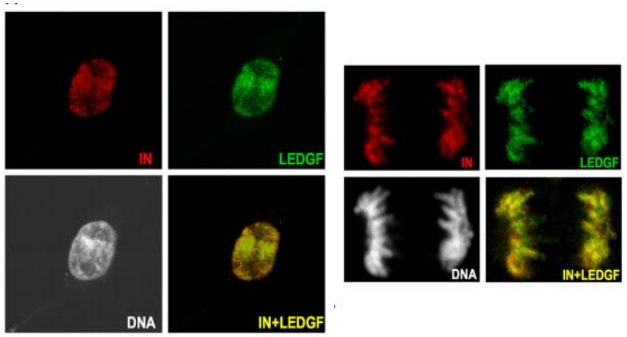
Several human proteins bind PICs:
BAF, HMGA, INI1, LEDGF, *etc.*

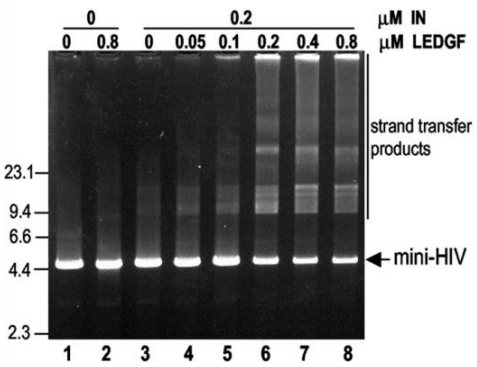
transcriptional co-activator LEDGF:

- Tightly binds HIV-1 integrase protein
- Tethers HIV-1 integrase to chromatin
- Depletion or knockout of cellular LEDGF results in reduction of HIV integration, residual integration events are essentially random

LEDGF/p75 tightly binds HIV-1 IN and stimulates integration!

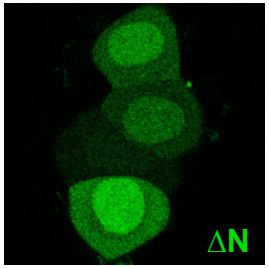




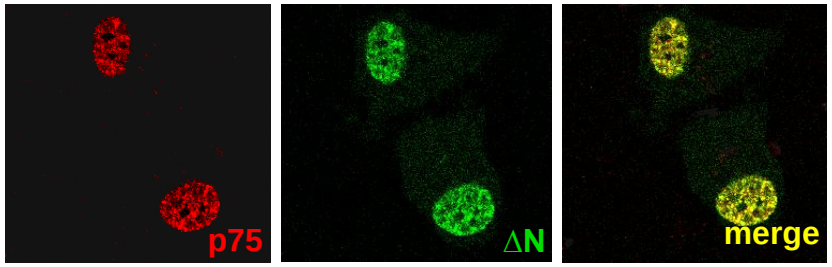


Cherepanov P. et al, JBC 2003.

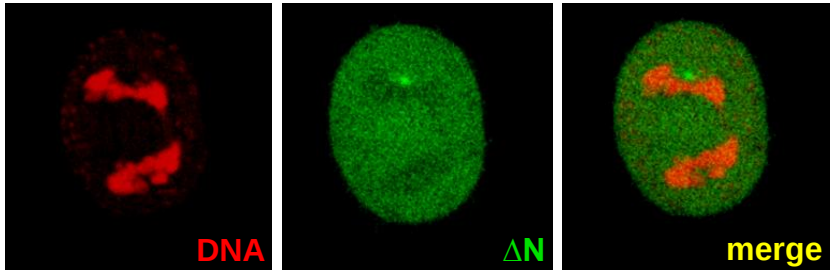
LEDGF/p75 tethers HIV-1 IN to chromatin!

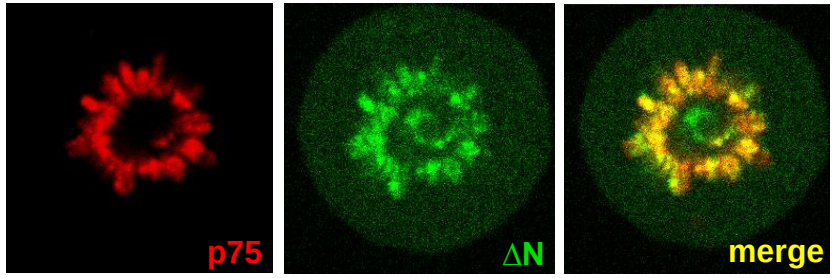


...becomes recruited to the nucleus



...and chromosomes upon over-expression of LEDGF

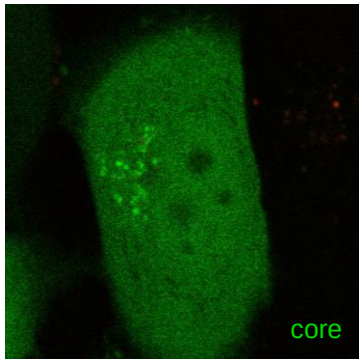


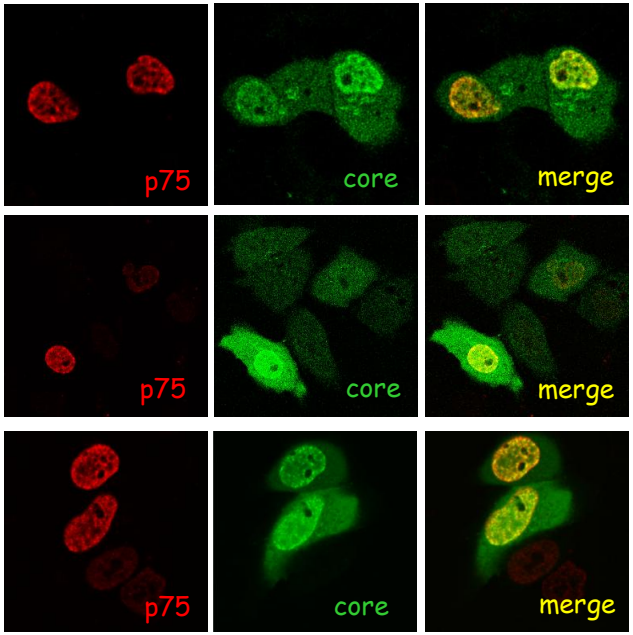


Maertens G. *et al.*, 2003, JBC

Maertens G.et al, JBC 2003.

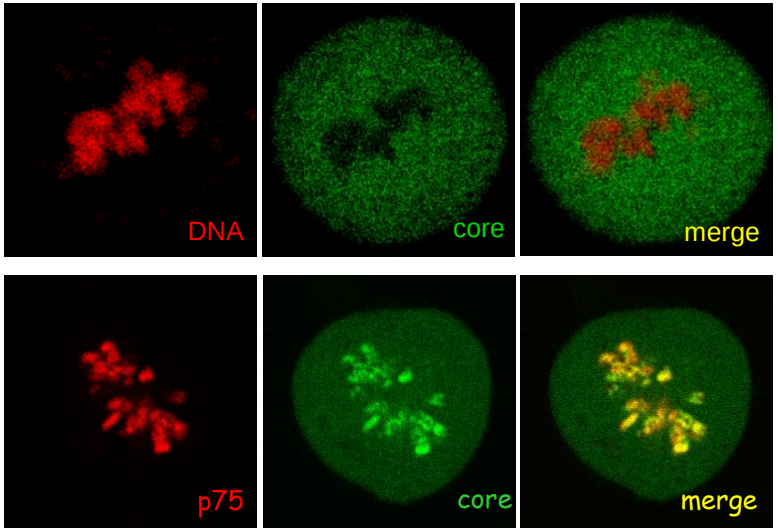
D D 35 E

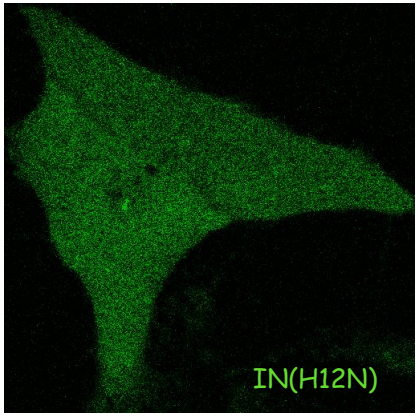


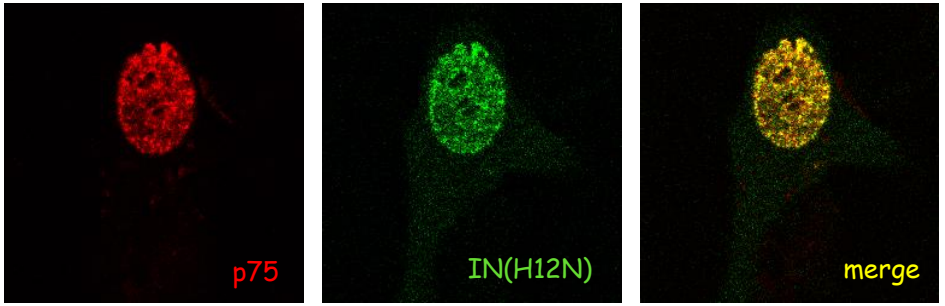


is recruited to the nucleus....

... and chromosomes upon over-expression of HcRed1-p75





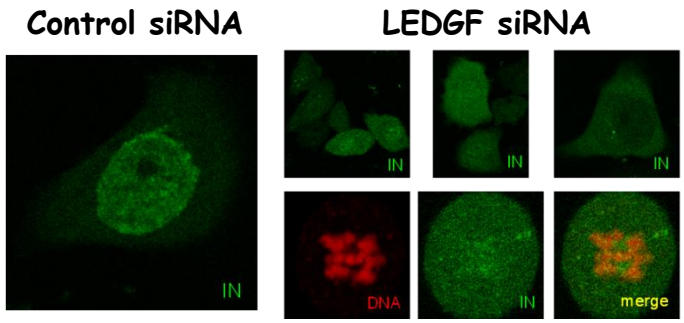


... Is distributed throughout the whole cell....

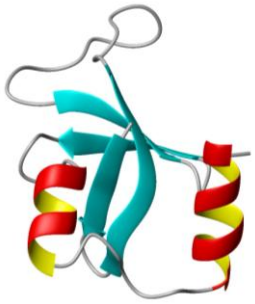
... But rescued upon over-expression of p75

Also in vitro, IN^{H12N} displays a greatly reduced affinity for LEDGF/p75

Maertens G. *et al.*, 2003, JBC


Control siRNA
LEDGF siRNA
IN
IN
IN
IN
DNA
IN
merge

Domain organization of LEDGF/p75

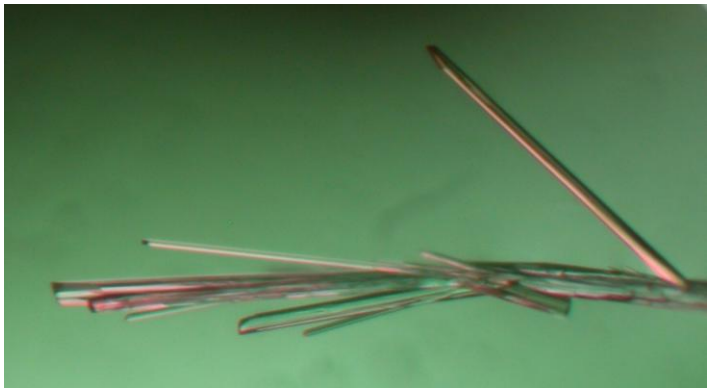


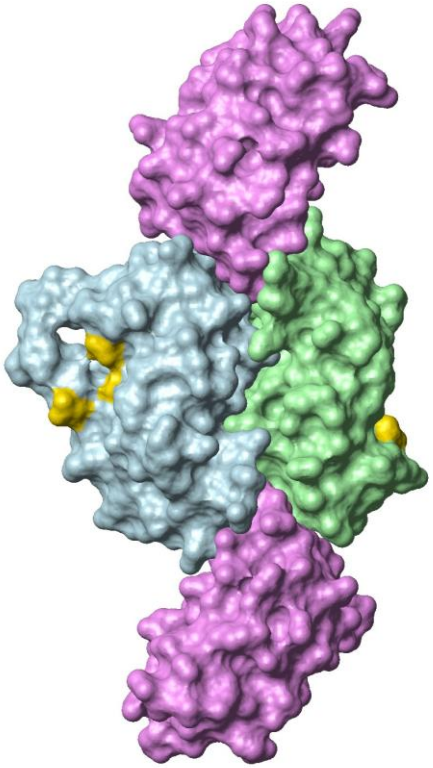
Association with chromatin (?)

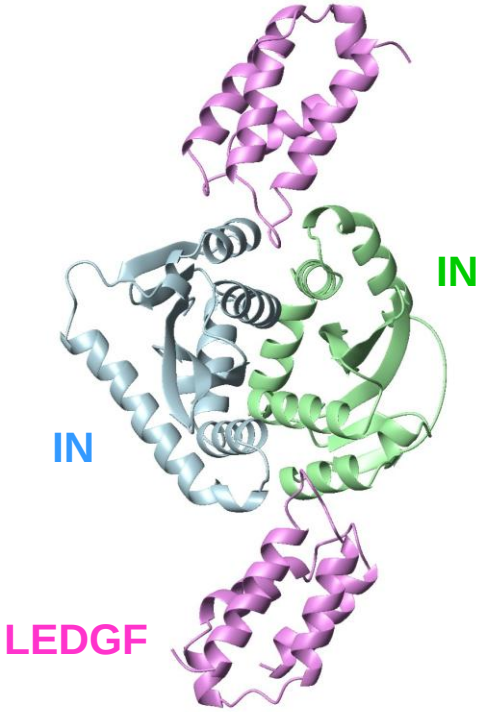
Protein interaction domain:

Lentiviral INs (HIV, FIV, etc), Transcription factors.

Structure

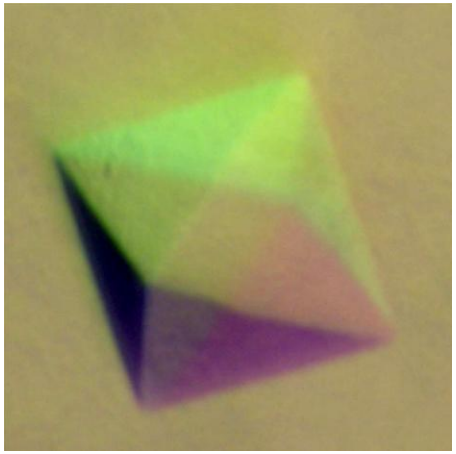


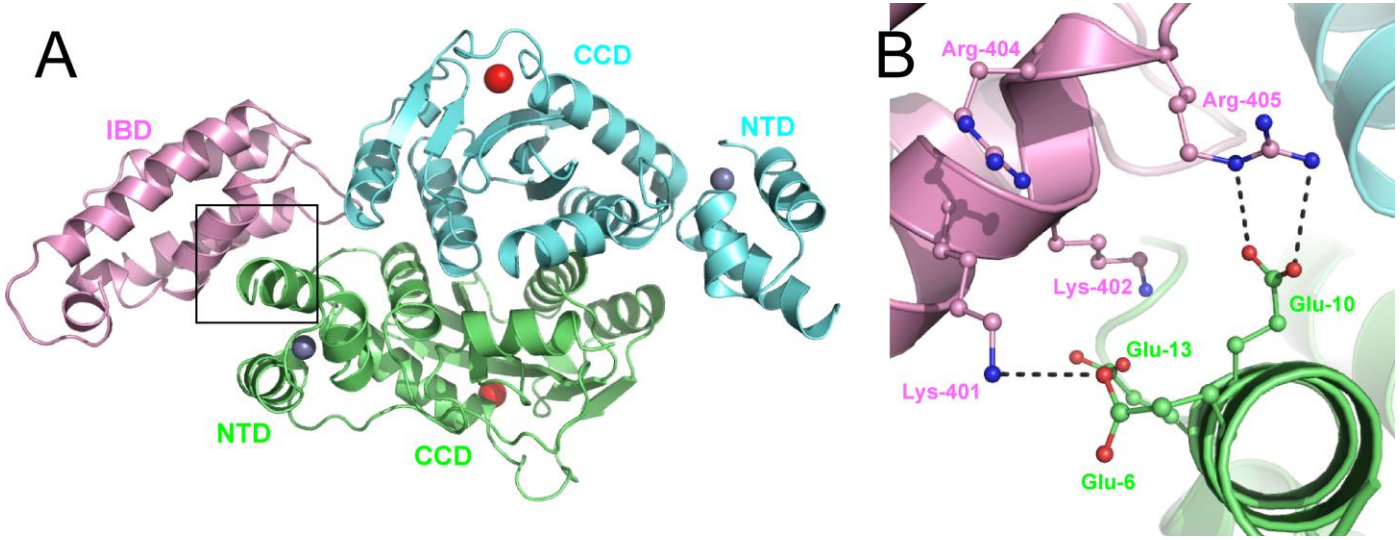




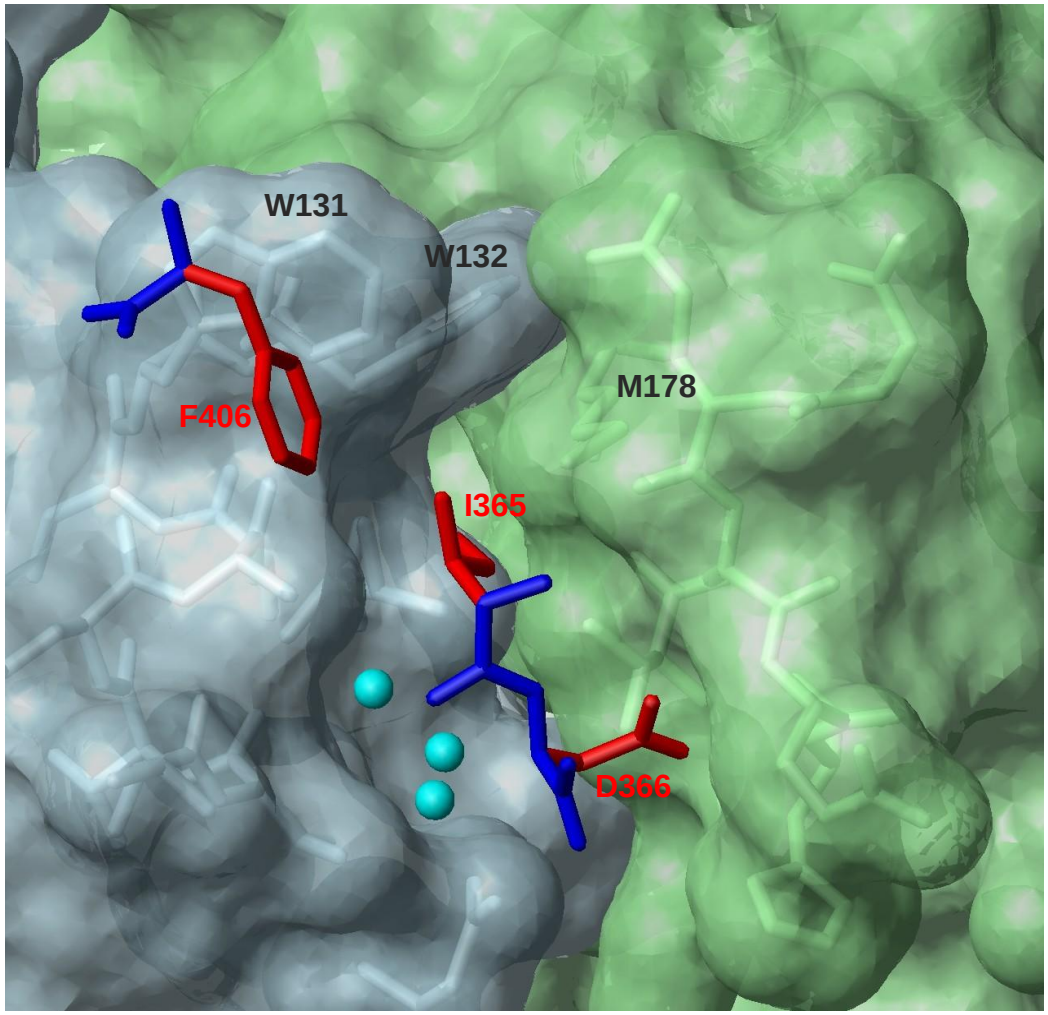
Hare S. et al, PLoS Path, 2009

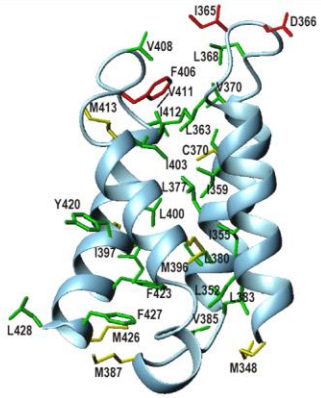
another structure ….



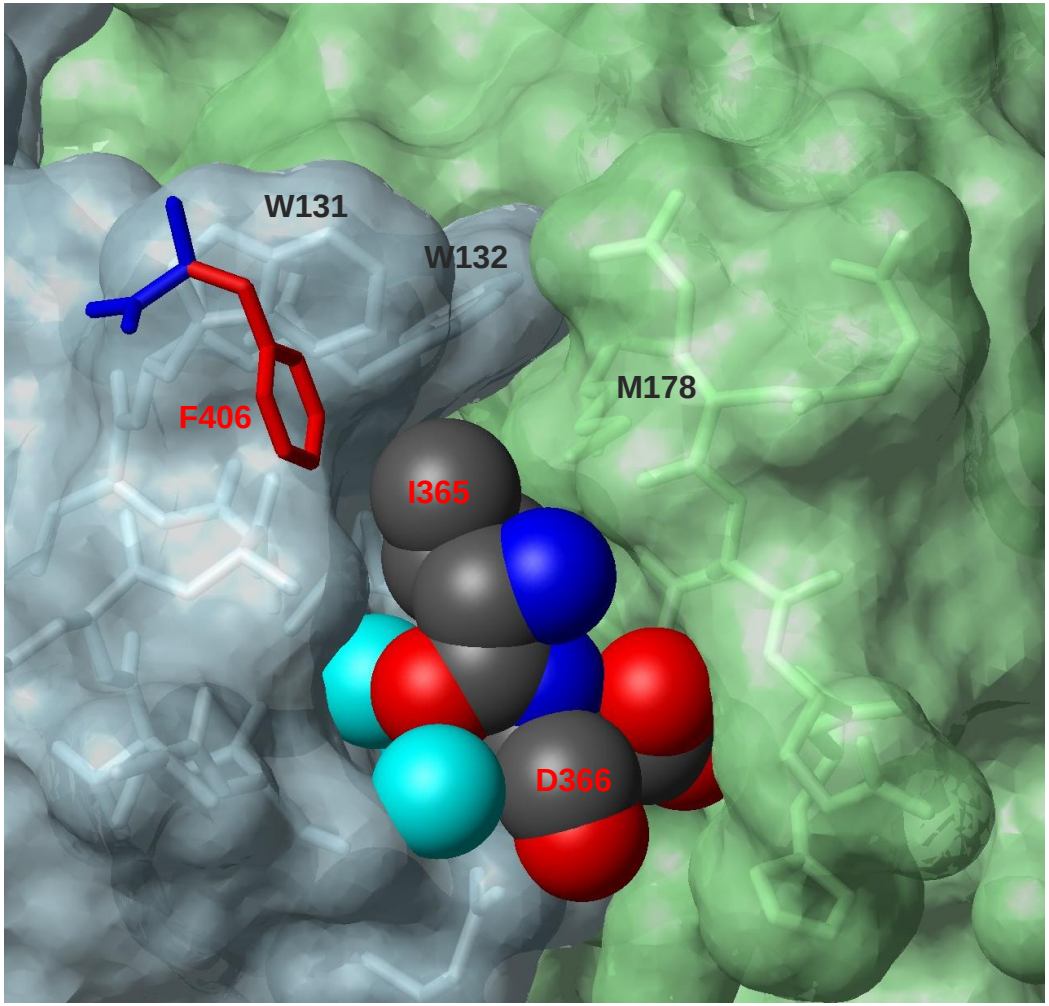


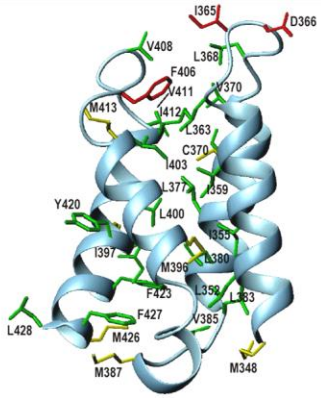
The roles of the LEDGF hot-spot residues in the recognition:



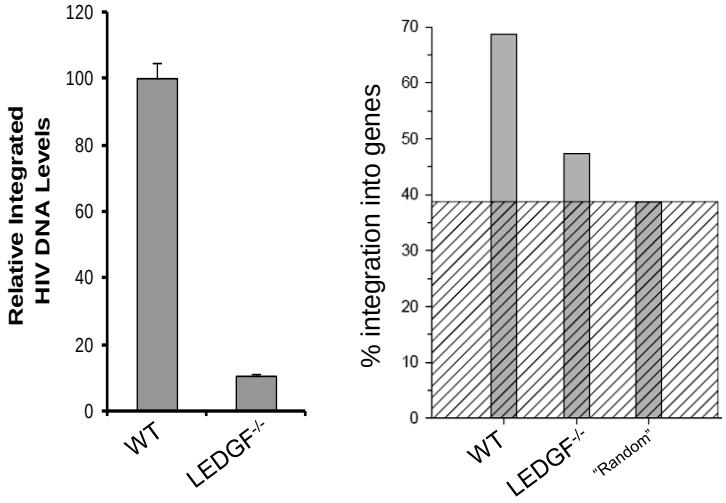


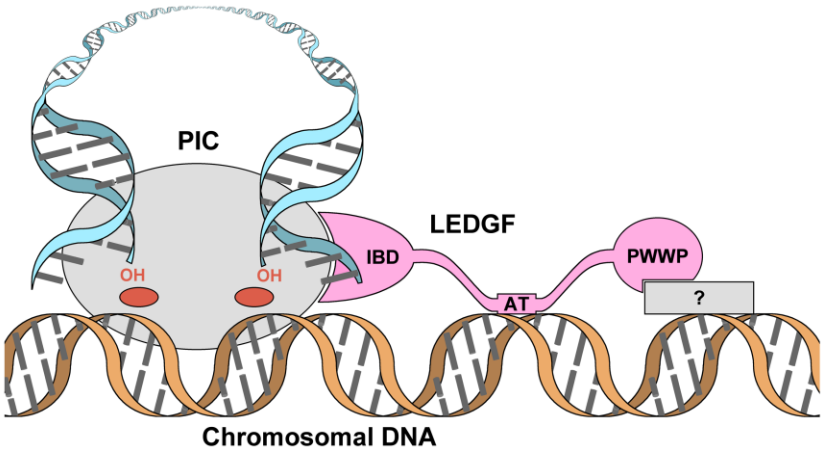
The roles of the LEDGF hot-spot residues in the recognition:





Loading of HIV PIC onto a target chromosome

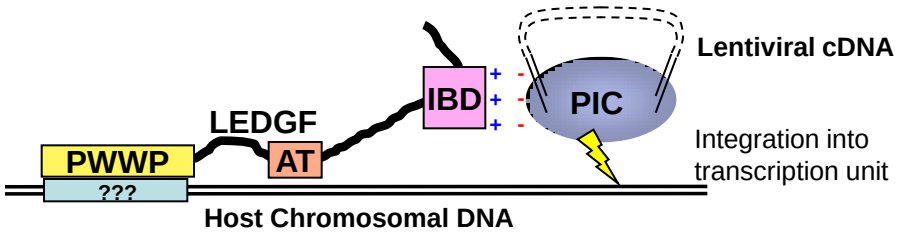




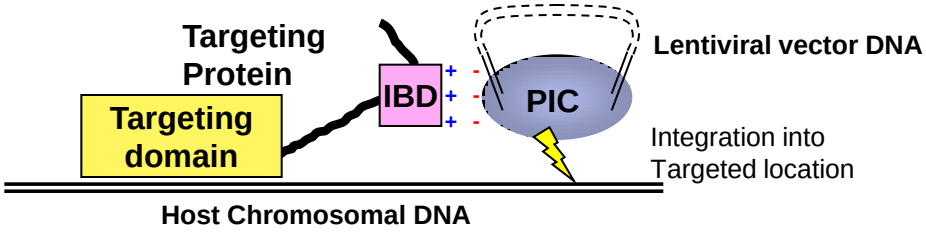
Hare S. and Cherepanov P, Viruses 2009

Targeting Application?

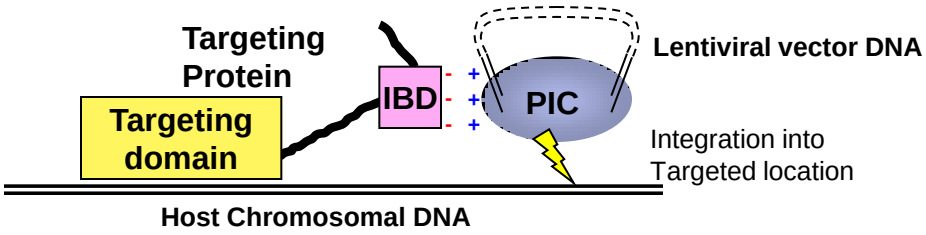
Native



Targeting



Specific targeting?



Facts to remember:

1) Integration is unique and obligatory step in retroviral replication.
2) IN inhibitors block HIV replication.
3) Retroviral INs are related to bacterial transposases.
4) On genomic scale, integration is not random: HIV and related lentiviruses favor transcription units; MLV likes promoter regions and CpG islands.
5) Integration depends on host cell factors and is directed to suitable loci by chromatin-bound host proteins.