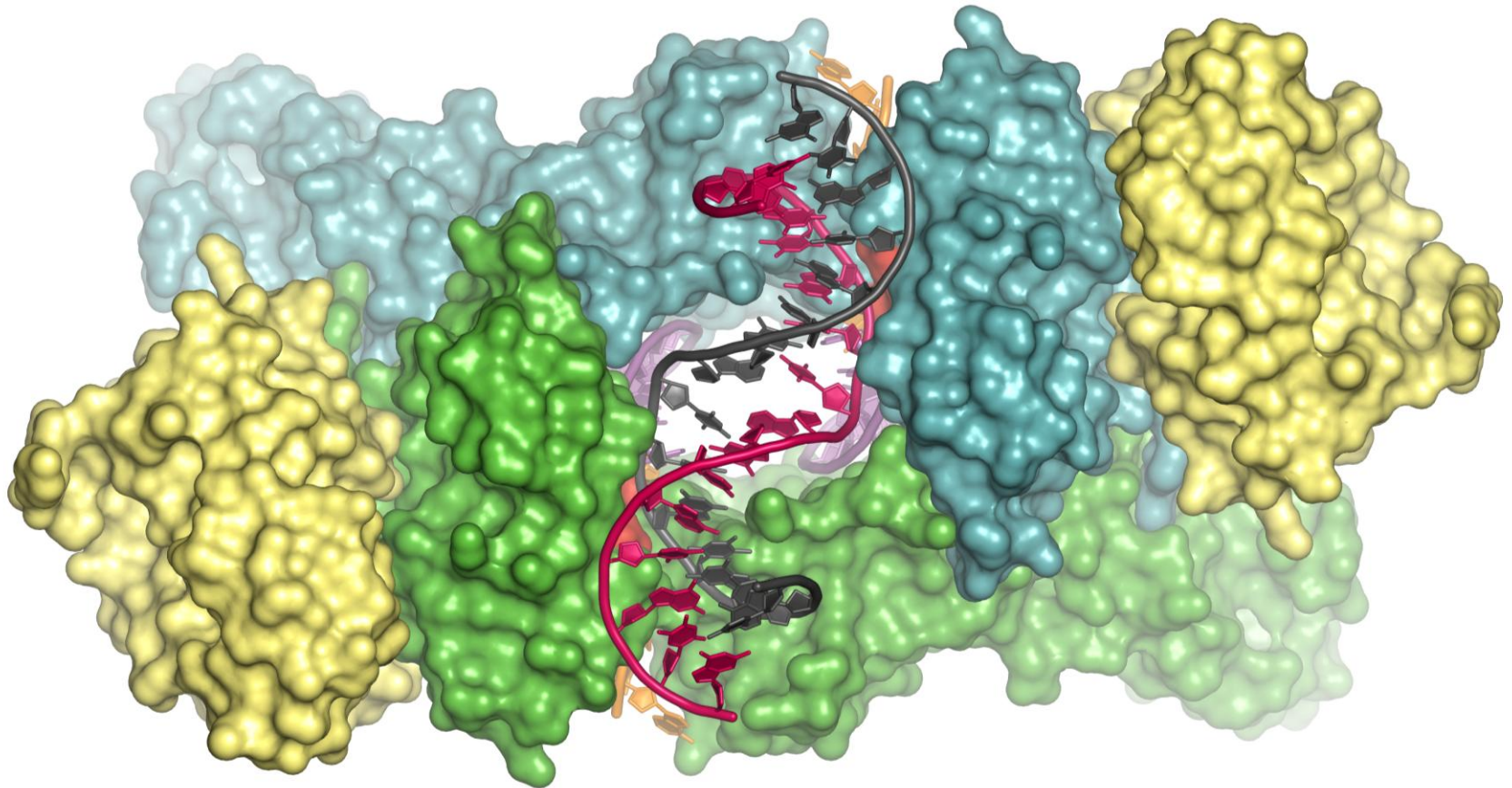
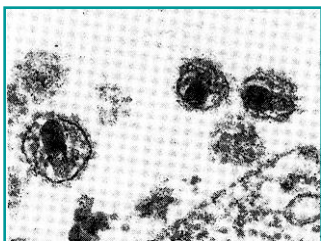
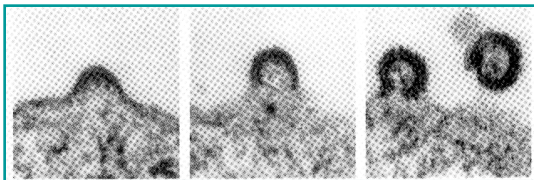
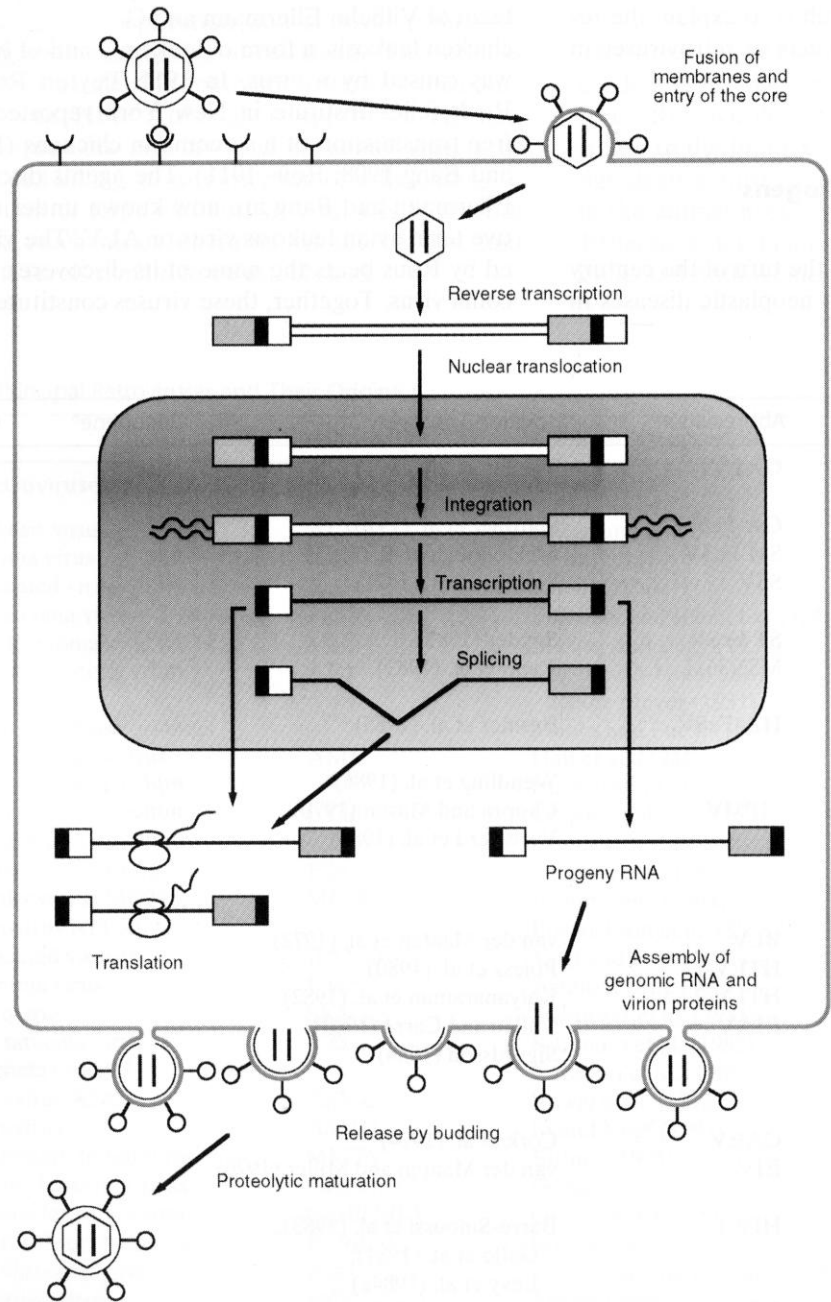


Retroviral DNA integration





Replication steps: Drug targets

1) Binding to CD4

~~2) Engagement of co receptor 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

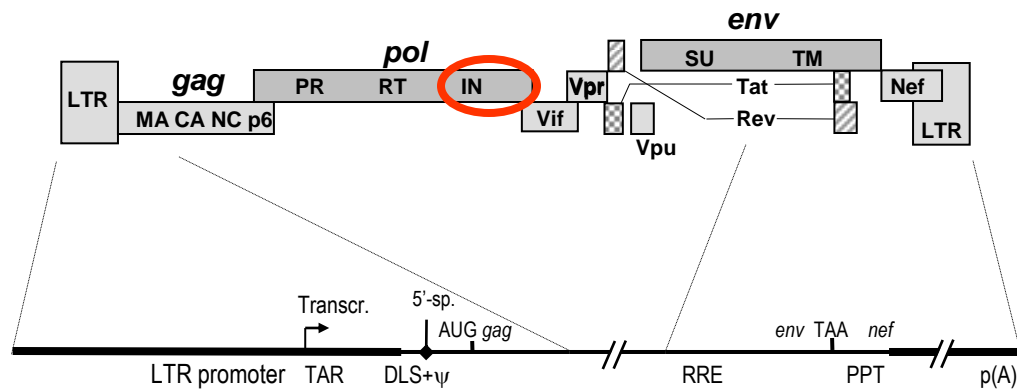
7) Budding

~~8) Protease-dependent maturation~~

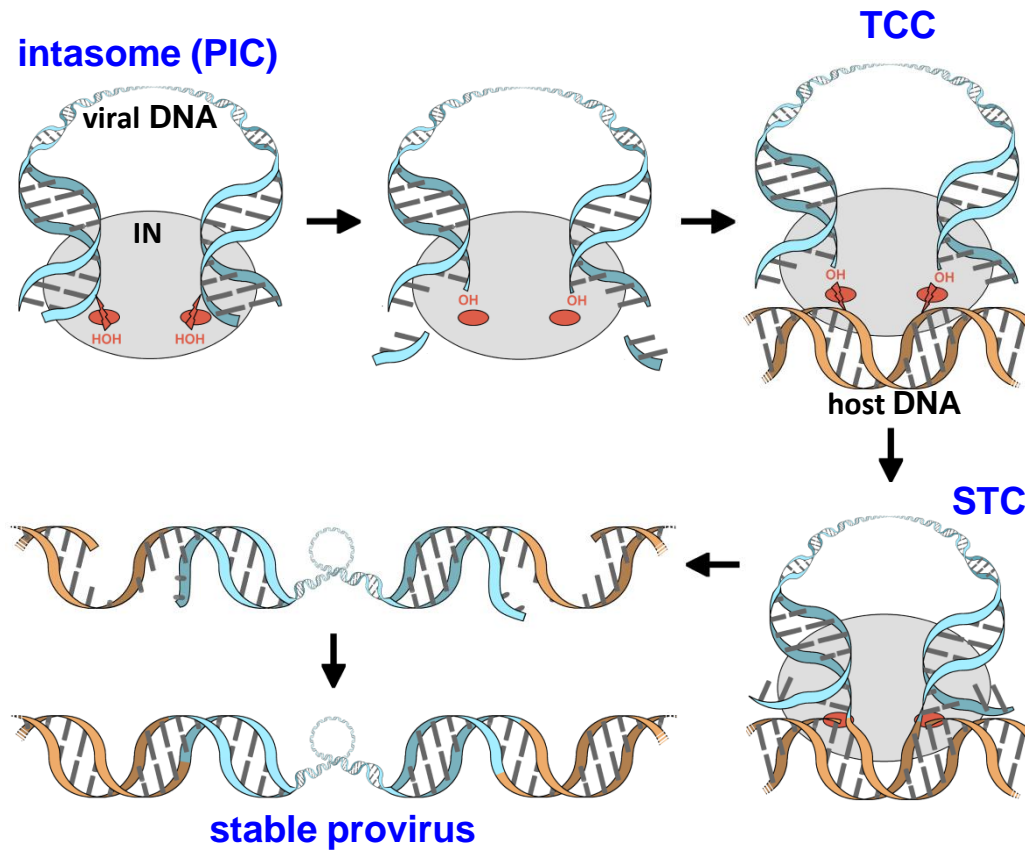
Clinically approved inhibitors:

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

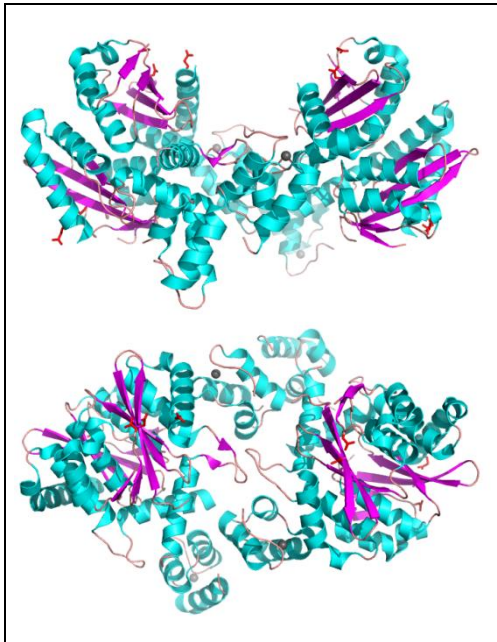
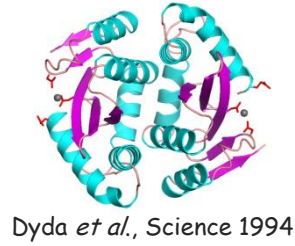
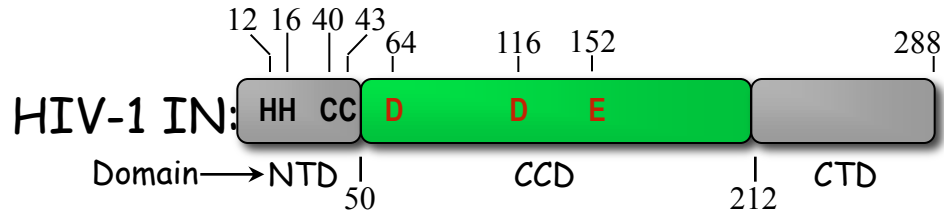
Genomic organization of HIV (proviral form, 9.7kb)



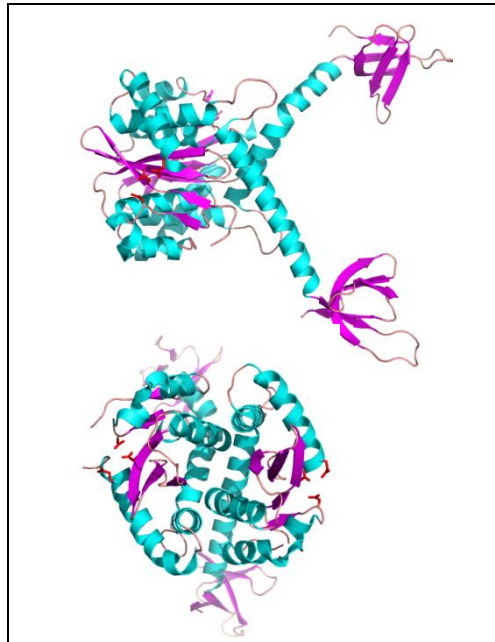
Catalytic functions of retroviral integrase



IN "shotgun crystallography": early work



Wang et al., EMBO J., 2001



Chen et al., PNAS, 2000

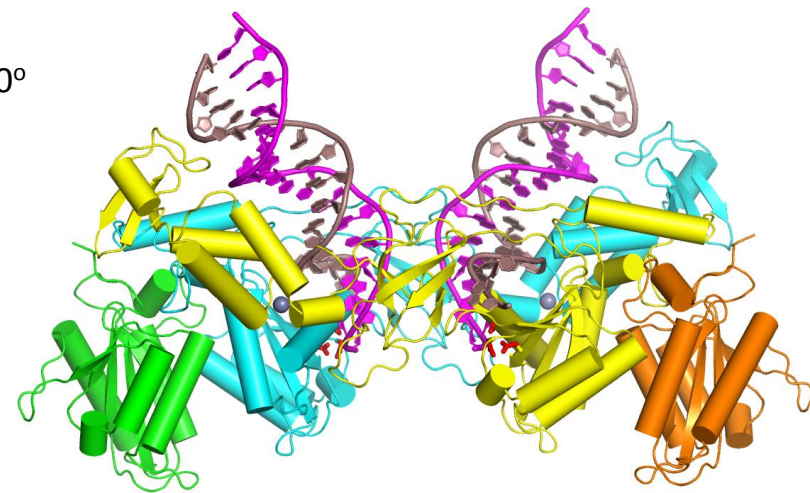


Composite models

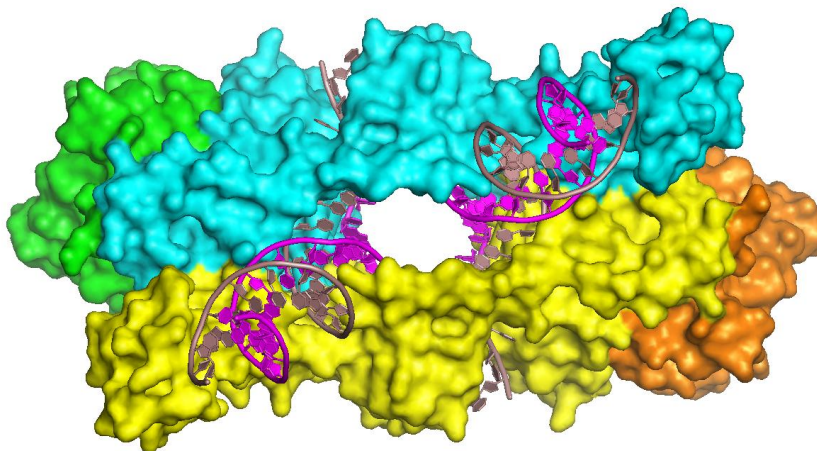
Overall architecture of the pre-integration complex (intasome)



90°

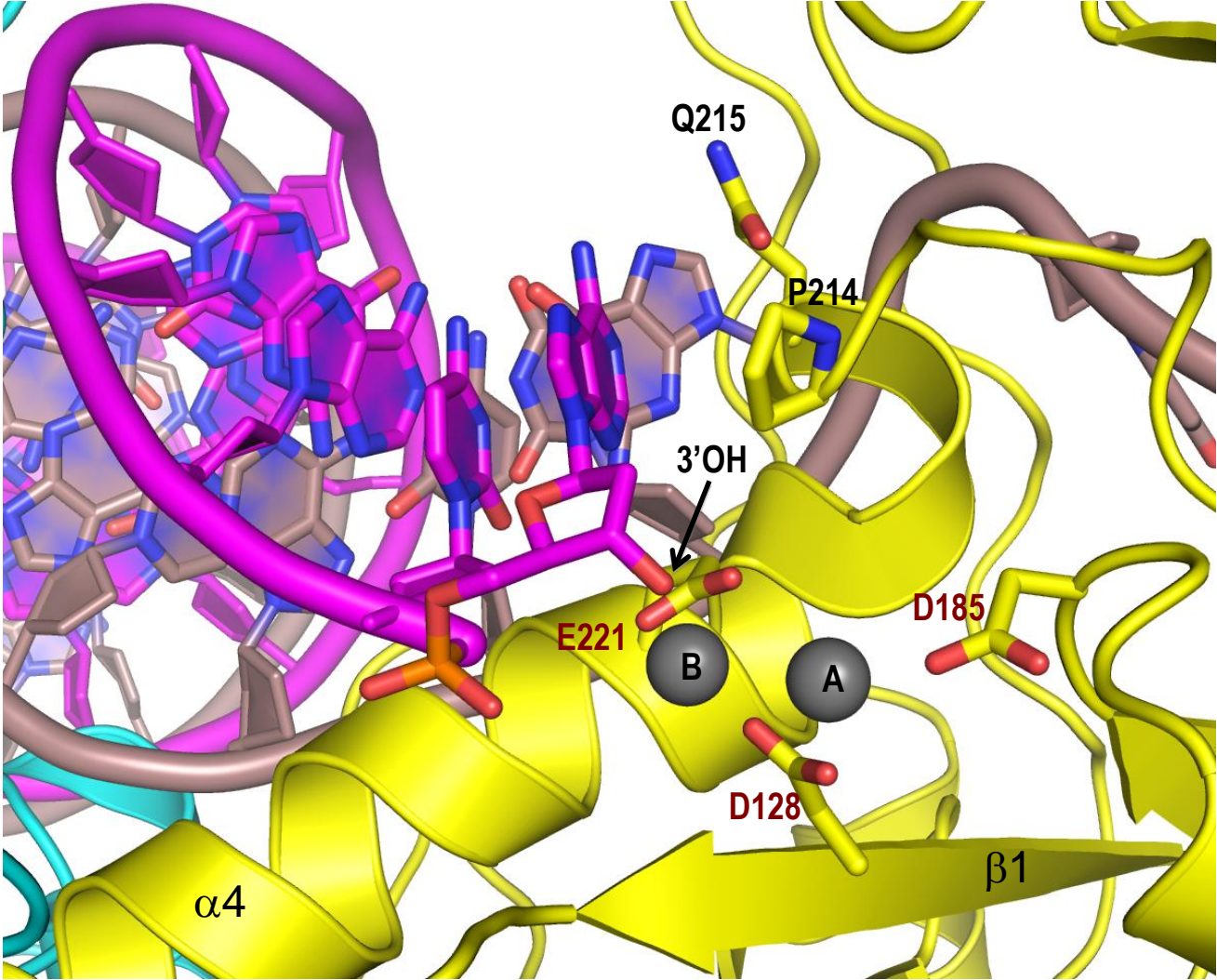


180°



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

Active site (prior to binding target DNA)



"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])

Retroviral
integrase
family

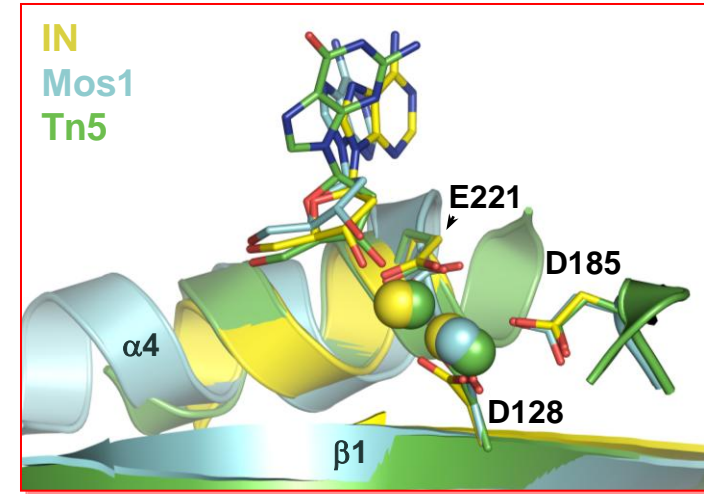
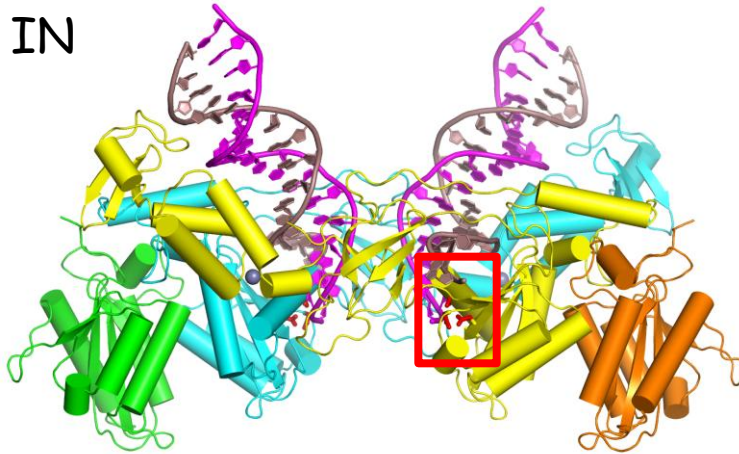
Retroviral integrases are NOT related to tyrosine
recombinases

Such as bacteriophage lambda *integrase*, P1 recombinase Cre,
yeast Flp recombinase, *etc*.

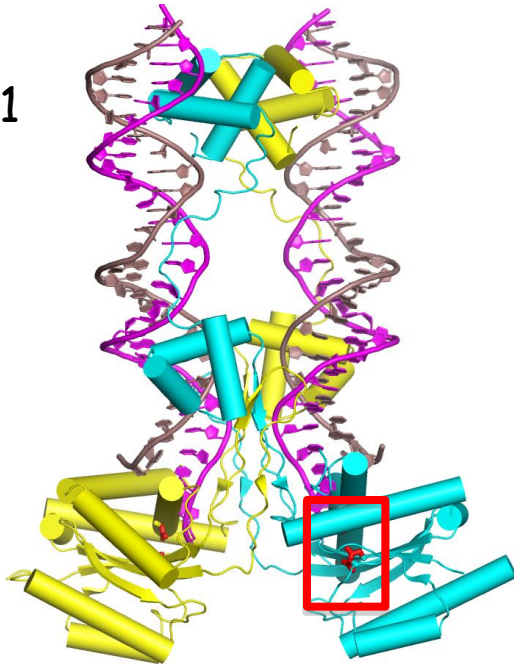
Int family

Comparison with another DDE(D) family members

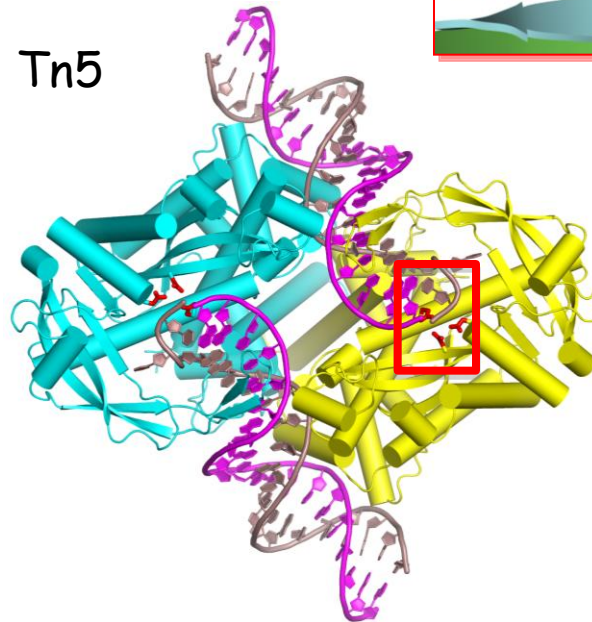
IN



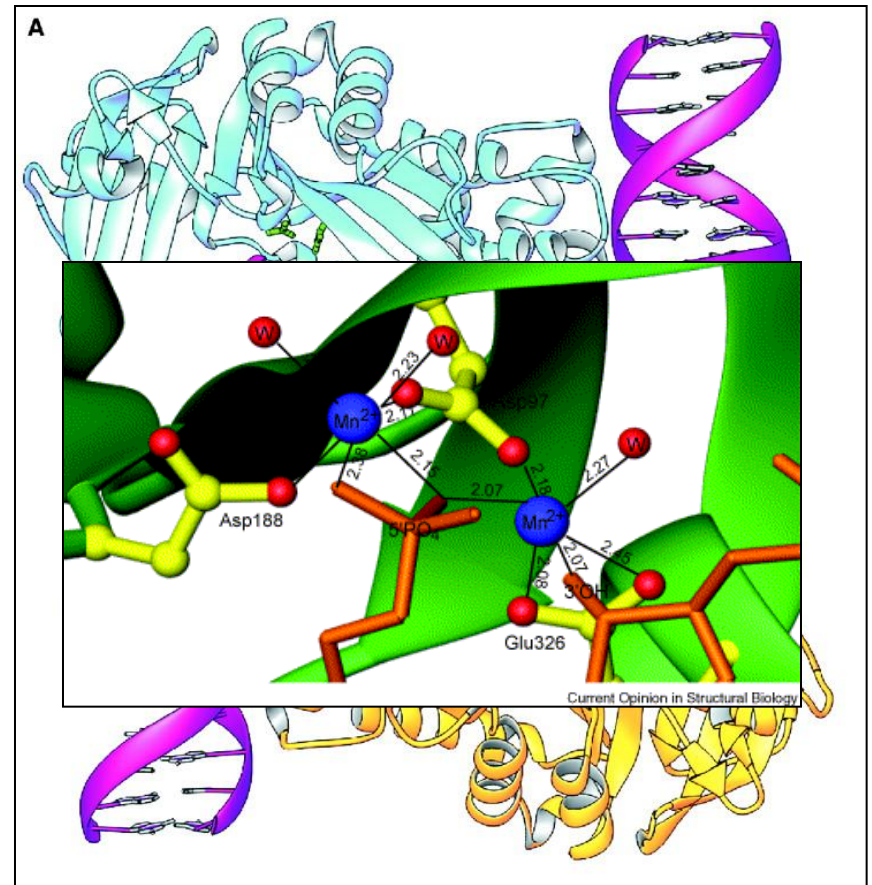
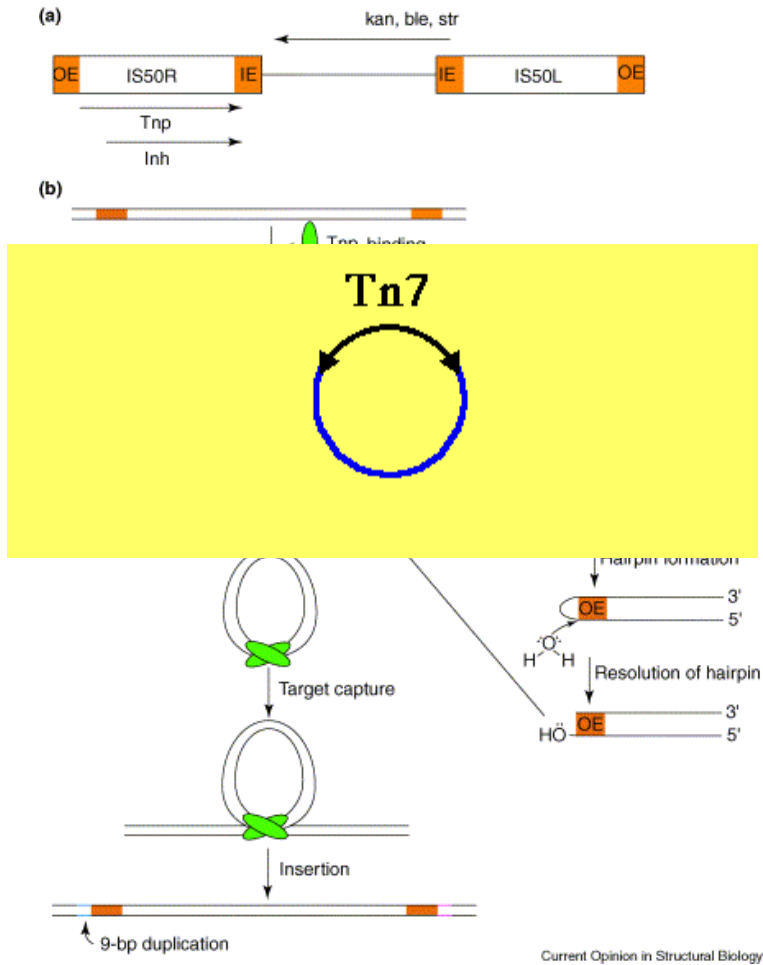
Mos1



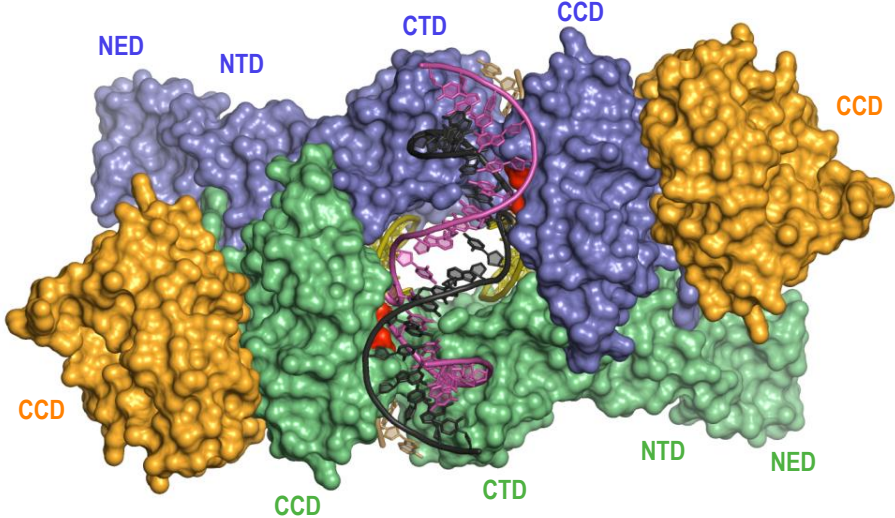
Tn5



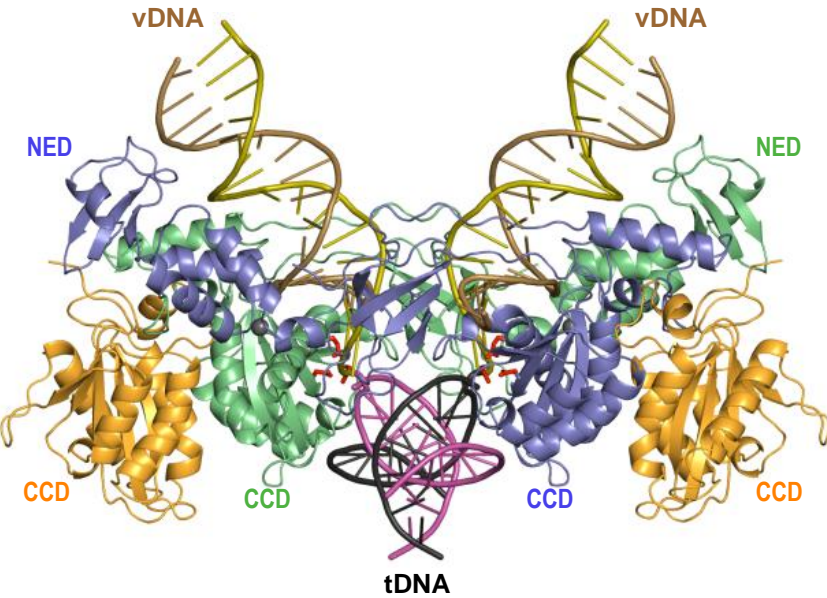
Prokaryotic transposases (Tn7, Tn5)



Strand transfer complex

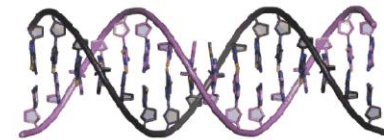
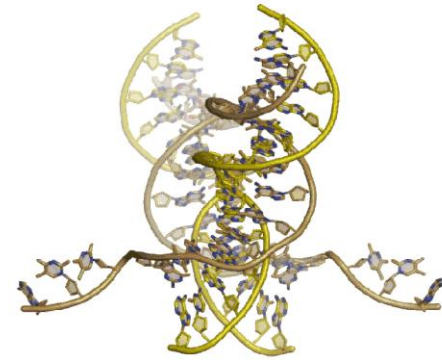
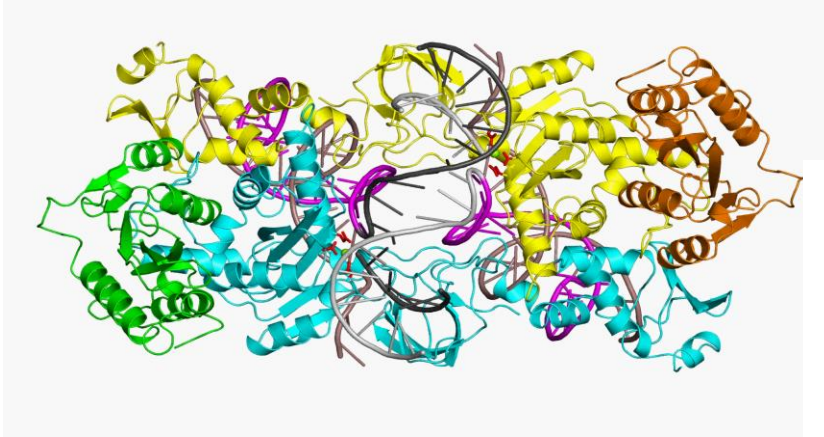


90°



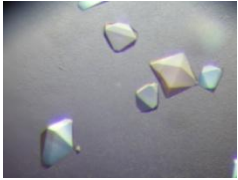
Active site mechanics during strand transfer

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

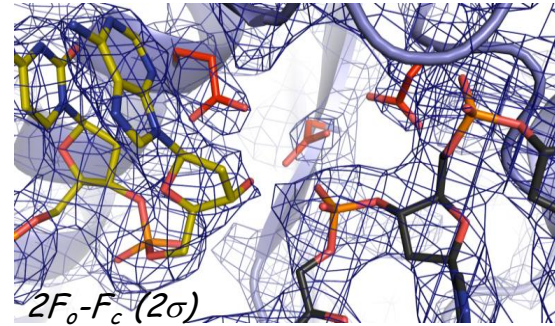


Strand transfer *in crystallo*

TCC_{EDTA}

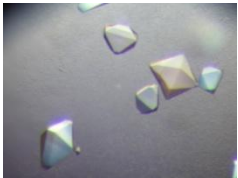


→ snap freeze

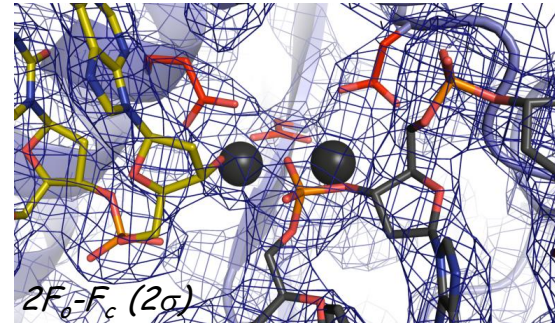


3.15 Å

TCC_{EDTA}

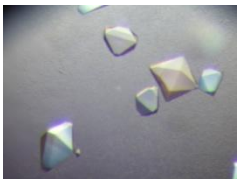


Soak in Mn²⁺
~ 90 sec → snap freeze

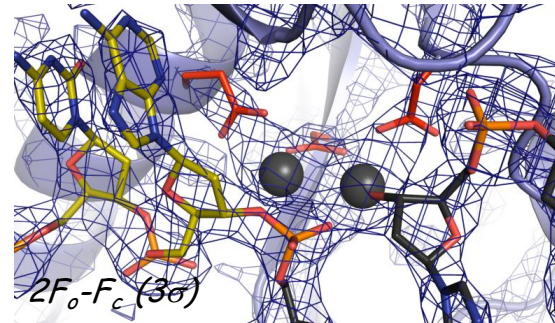


3.05 Å

TCC_{EDTA}

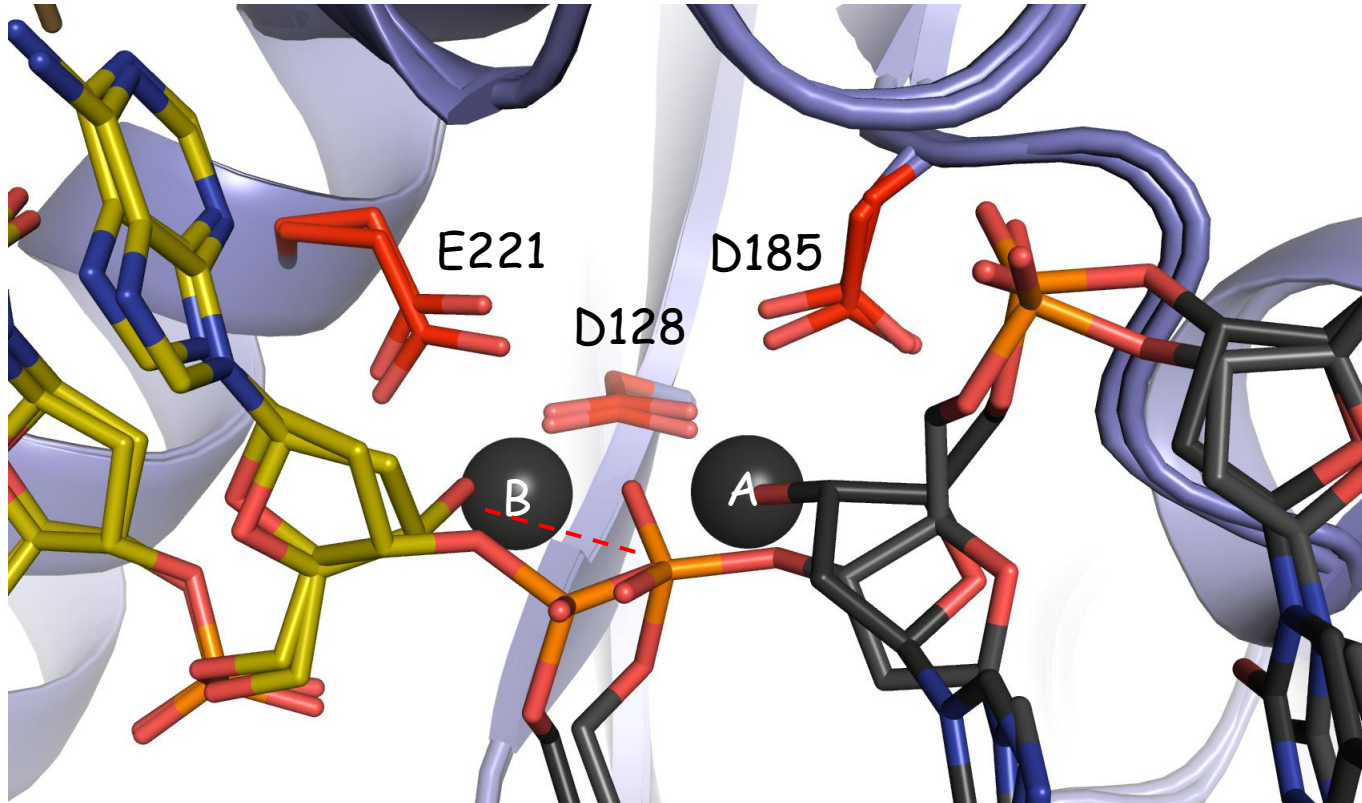


Soak in Mn²⁺
~ 3 min → snap freeze



3.00 Å

Structural change in active site renders integration reaction irreversible



Clinical InSTIs:

MK-0518 (FDA approved) Merck

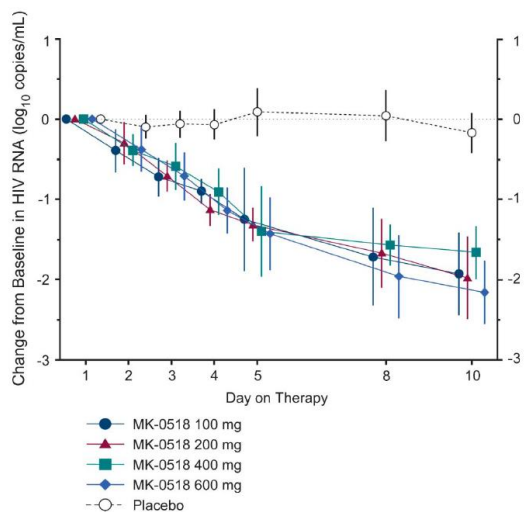
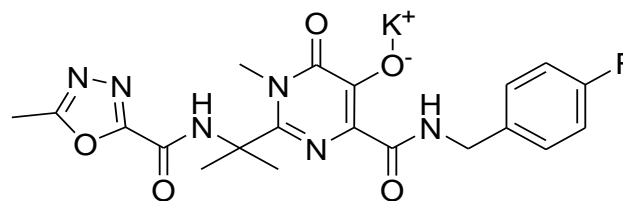
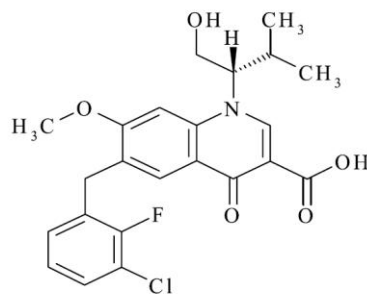


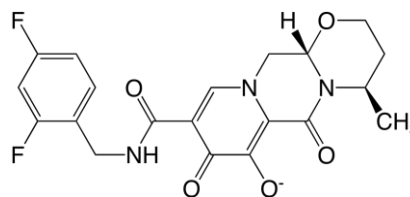
FIGURE 2. Change from baseline at day 10 in \log_{10} HIV RNA (with 95% CI). Error bars indicate 95% CIs. Data for a total of 7 patients (from 4 of the 5 treatment groups) were not available on day 5.



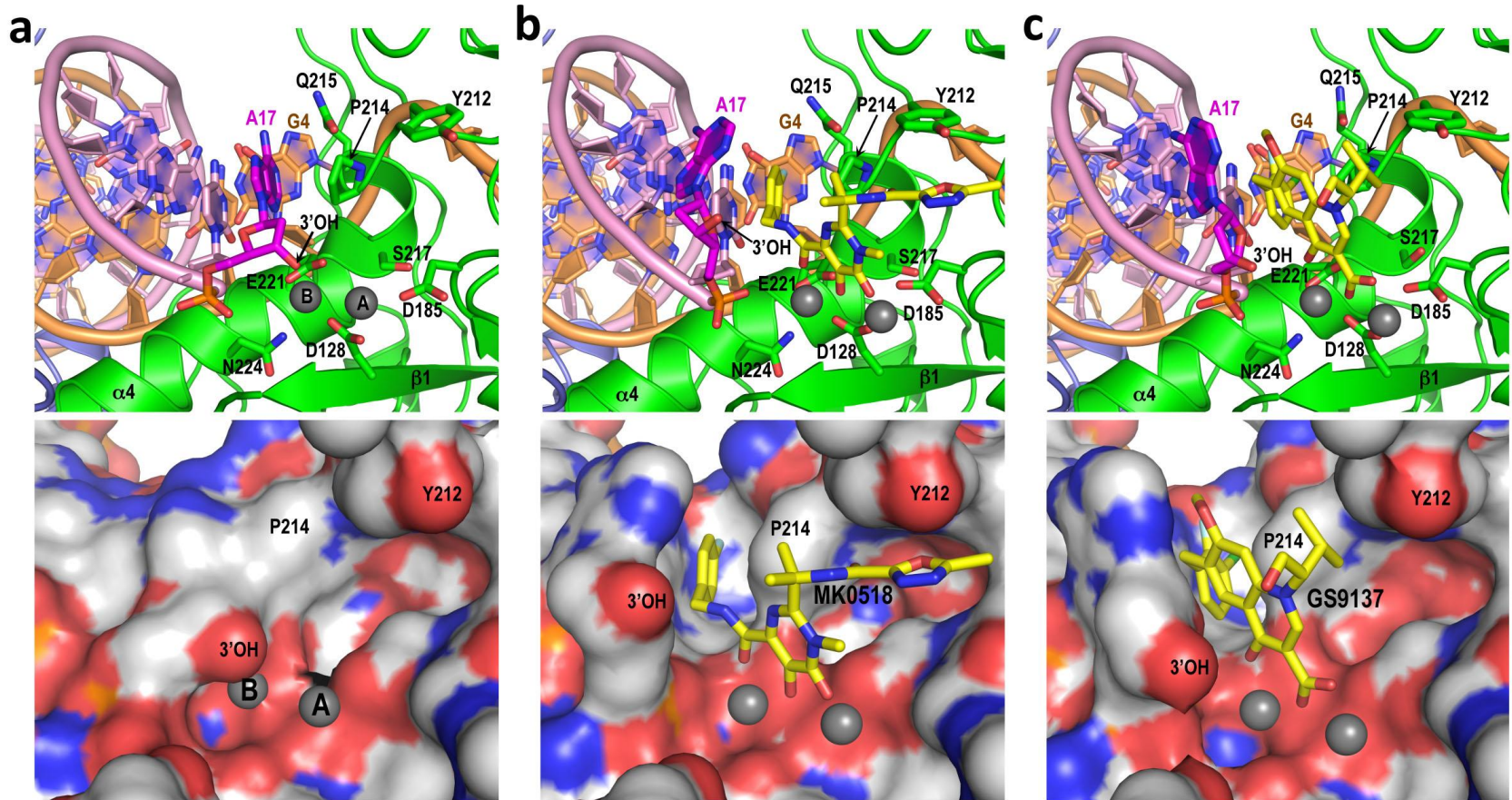
GS-9137 (phase III), Gilead



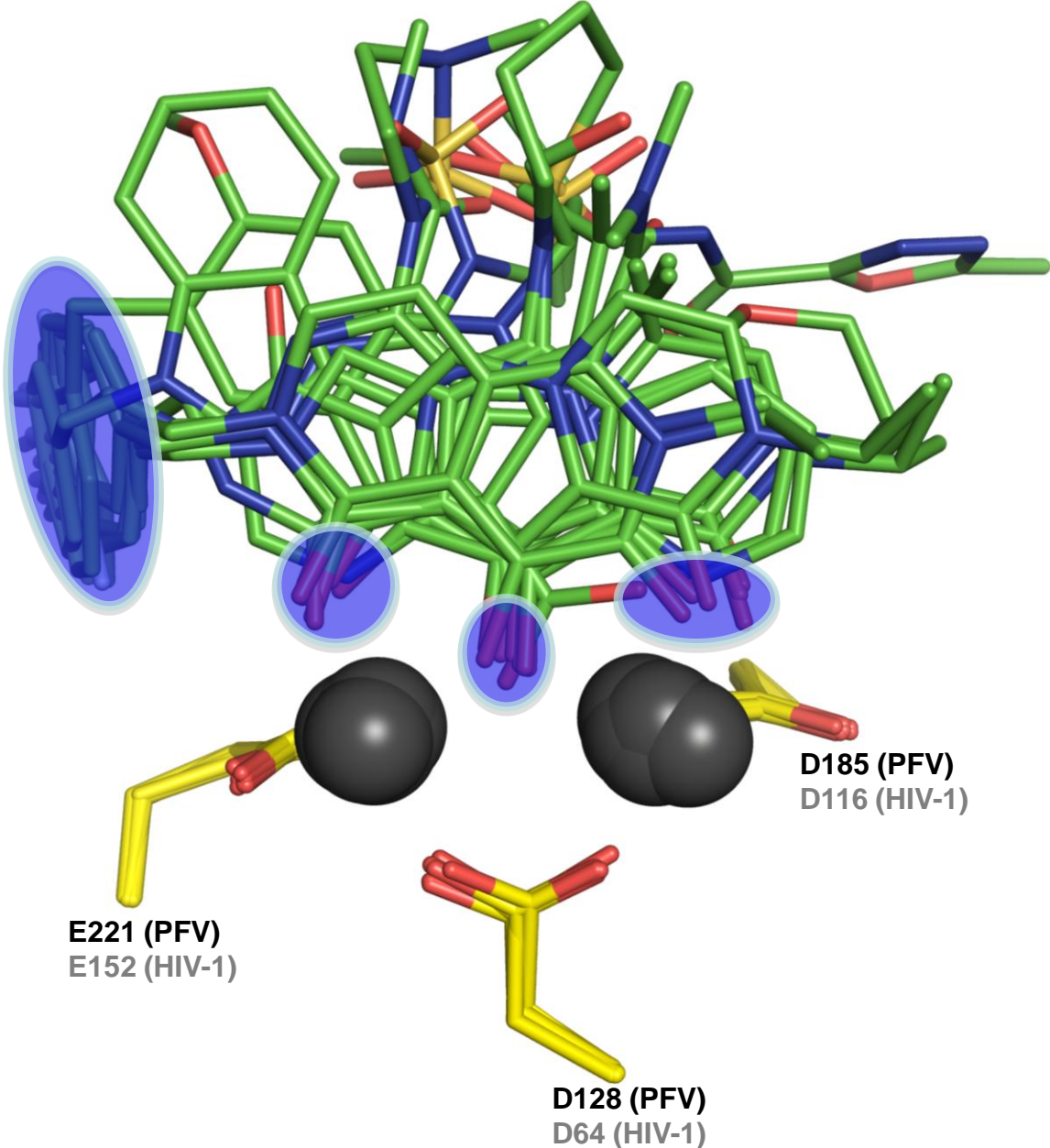
GSK-572 (phase II) Glaxo



Mechanism of InSTI action (a)

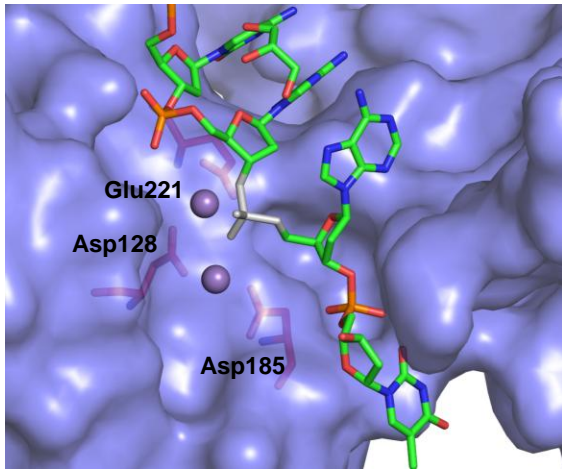


Consensus INSTI pharmacophore

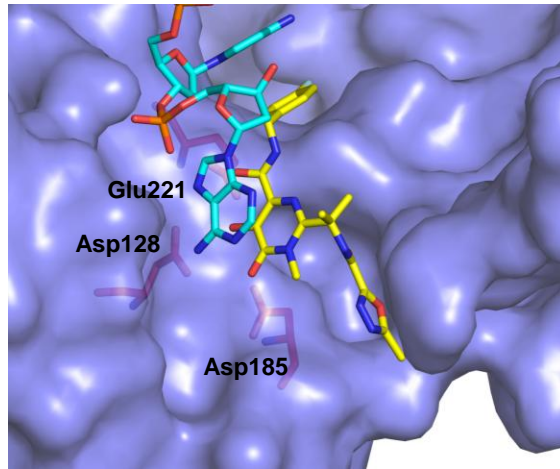


Mechanism of InSTI action (b)

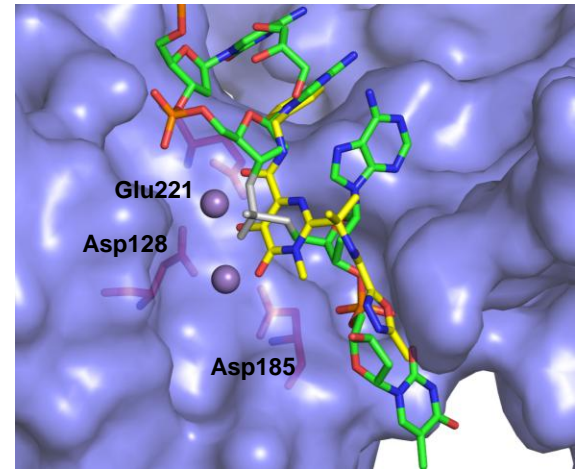
Intasome (Uncleaved substrate)



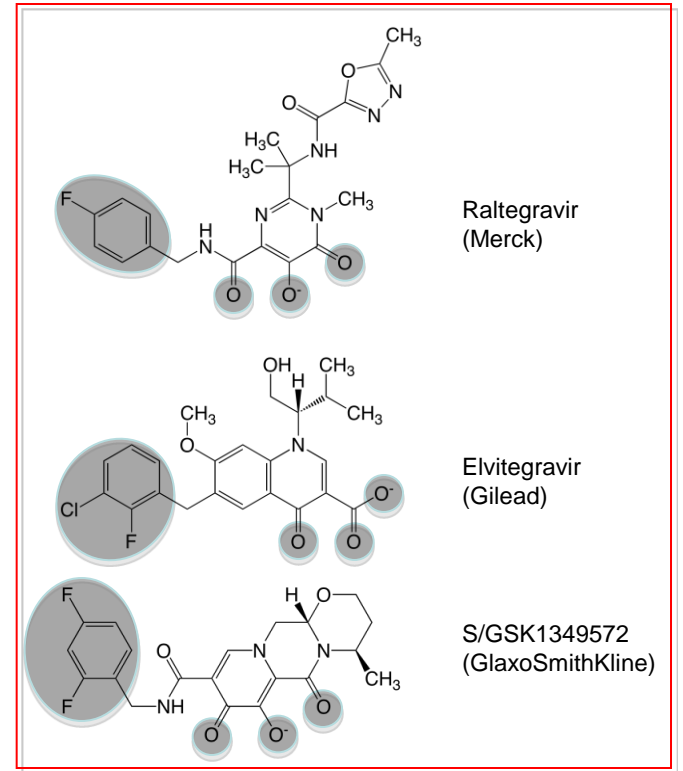
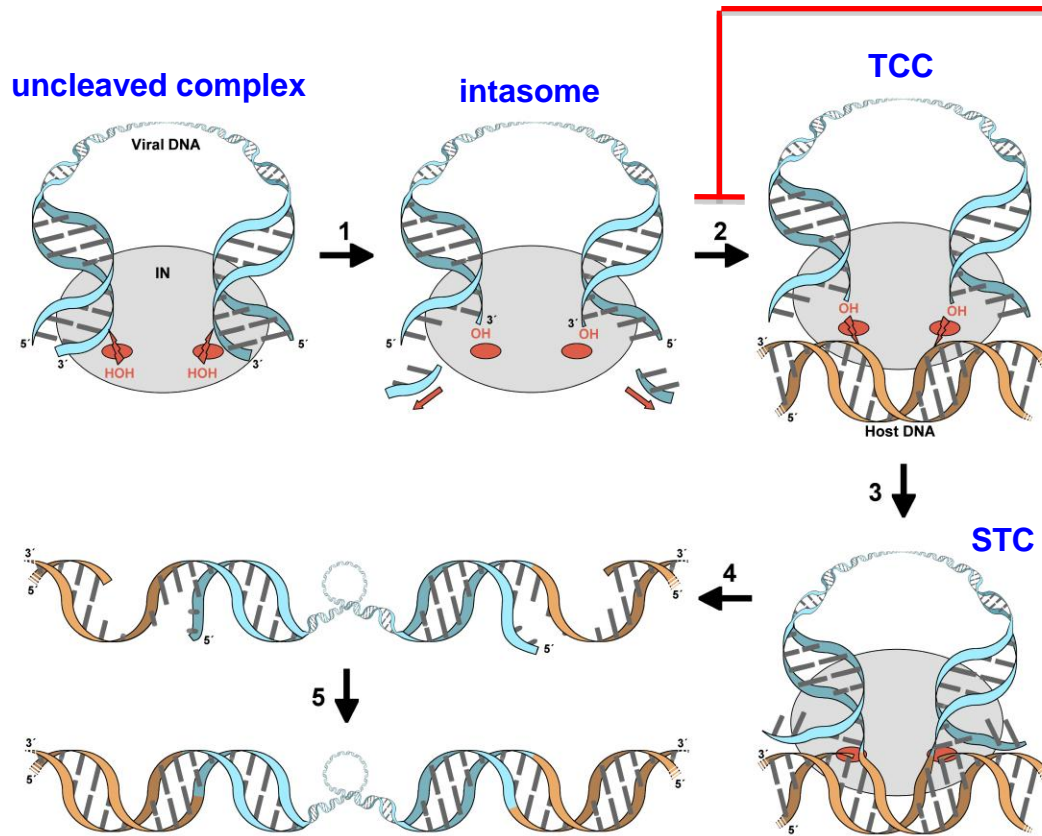
Intasome (Cleaved substrate, RAL)



Overlay



Retroviral integration

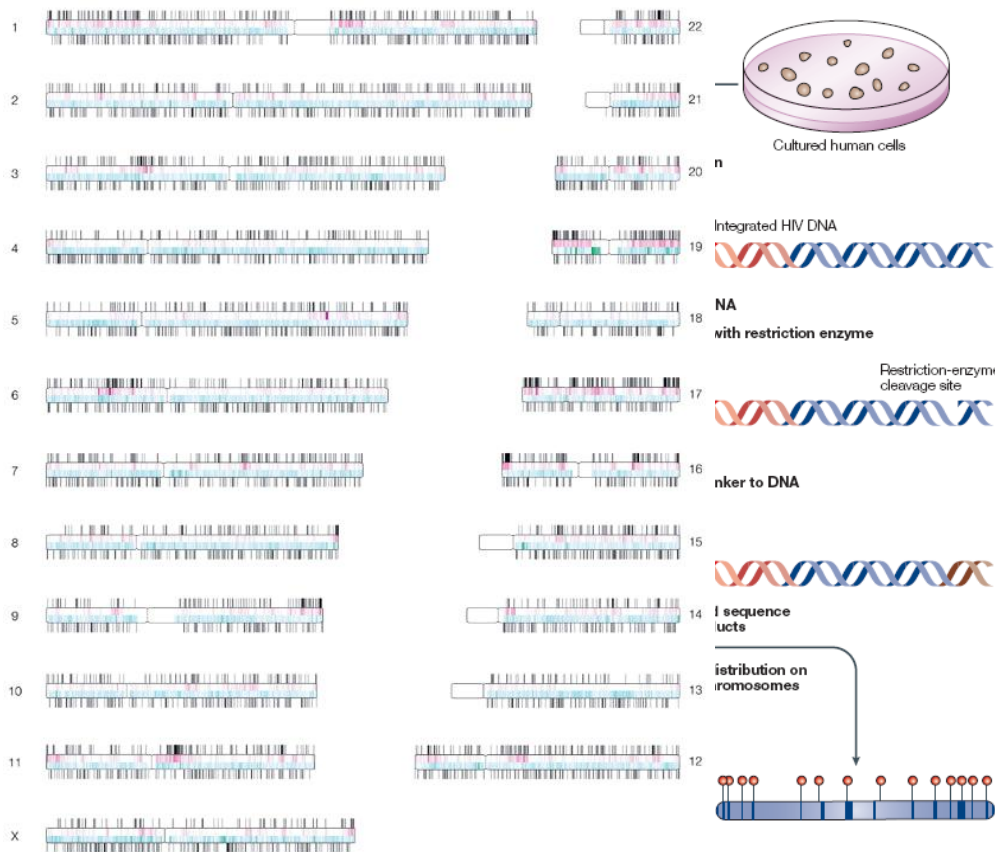


INSTI's:

- Bind to intasome
- After 3' processing (final dinucleotide needs to be removed)
- "flip" final nucleotide away from active site
- Prevent strand transfer

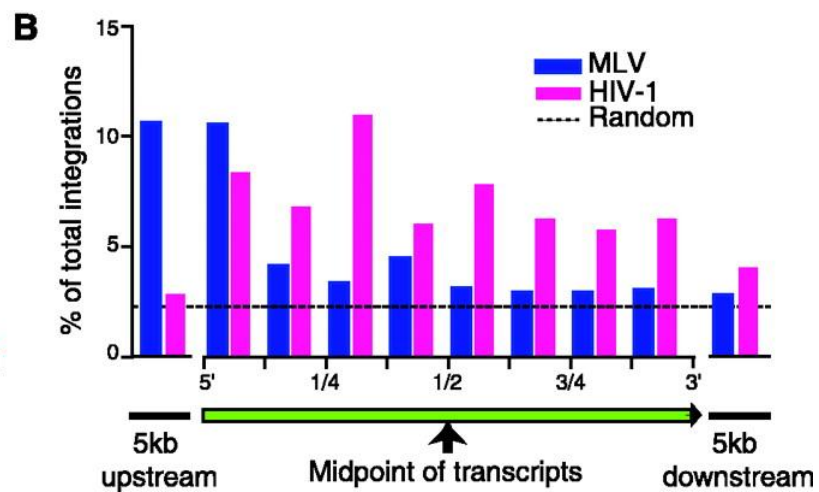
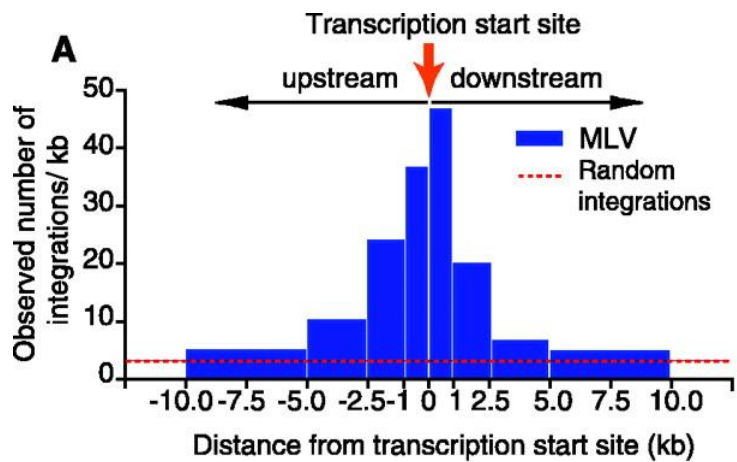
Do retroviruses favor certain chromosomal loci for integration?

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



Target site selection by yeast retrotransposons

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

“tethering model”

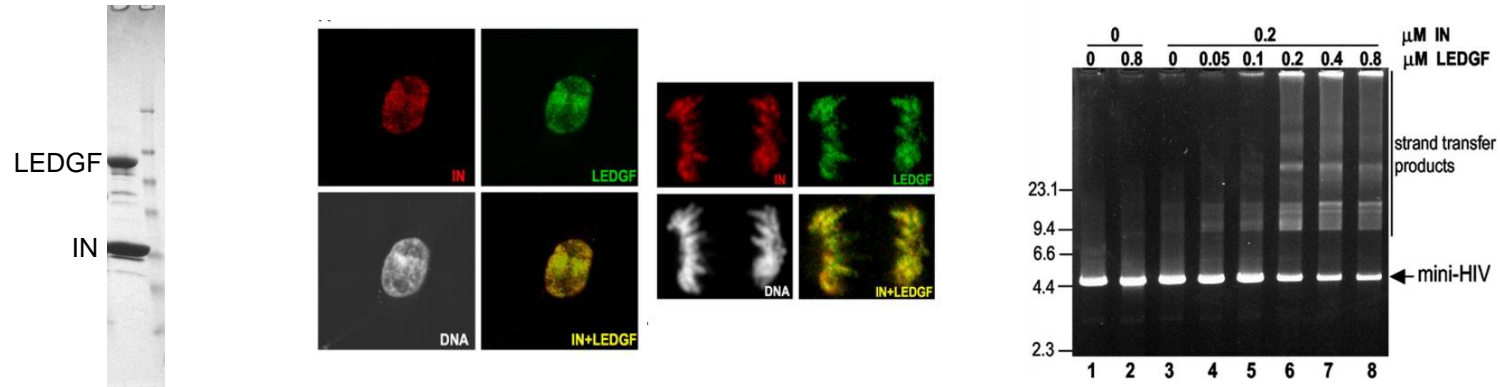
Any targeting factors for HIV?

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

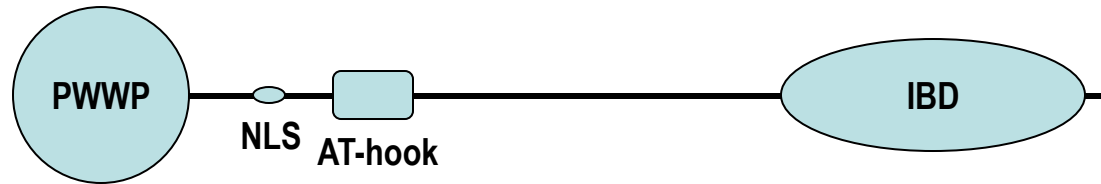
transcriptional co-activator LEDGF:

- 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!



Domain organization of LEDGF/p75



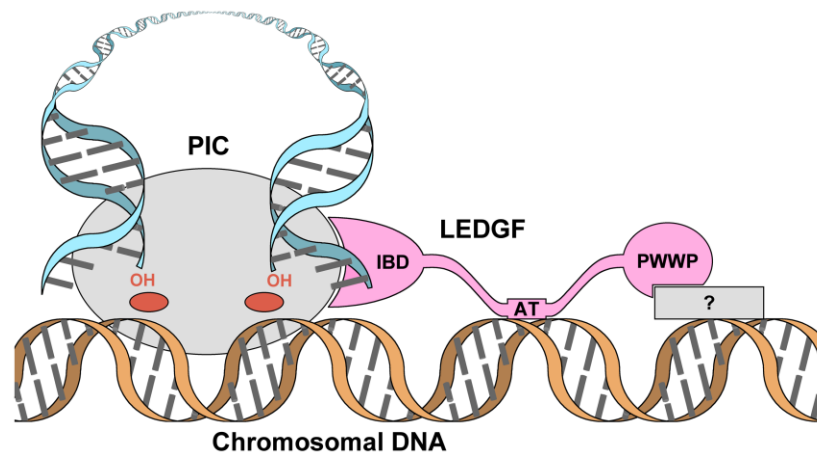
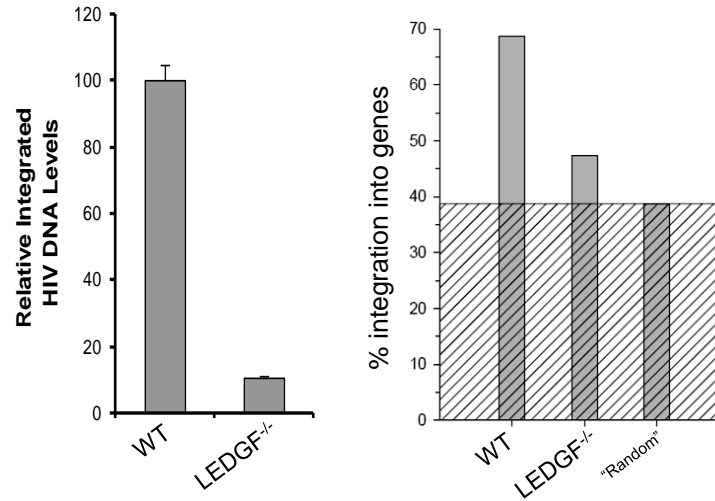
Association with chromatin
(?)



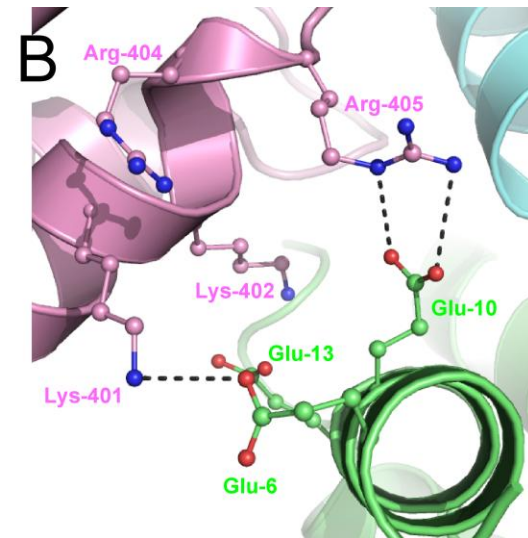
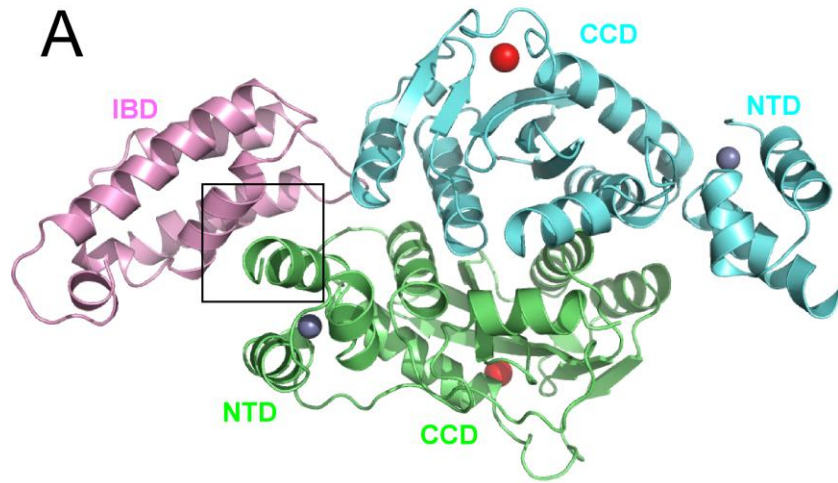
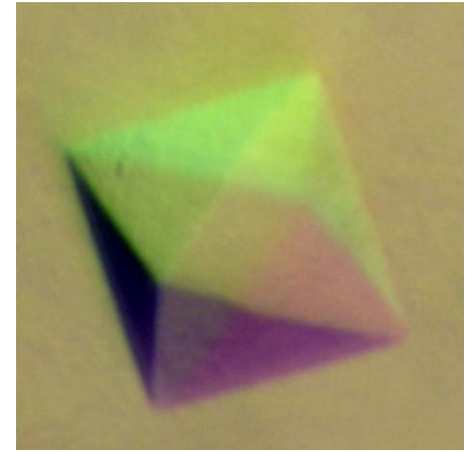
Protein interaction domain:

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

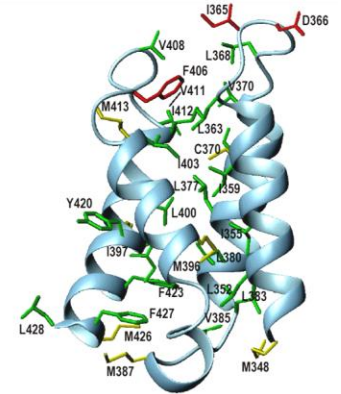
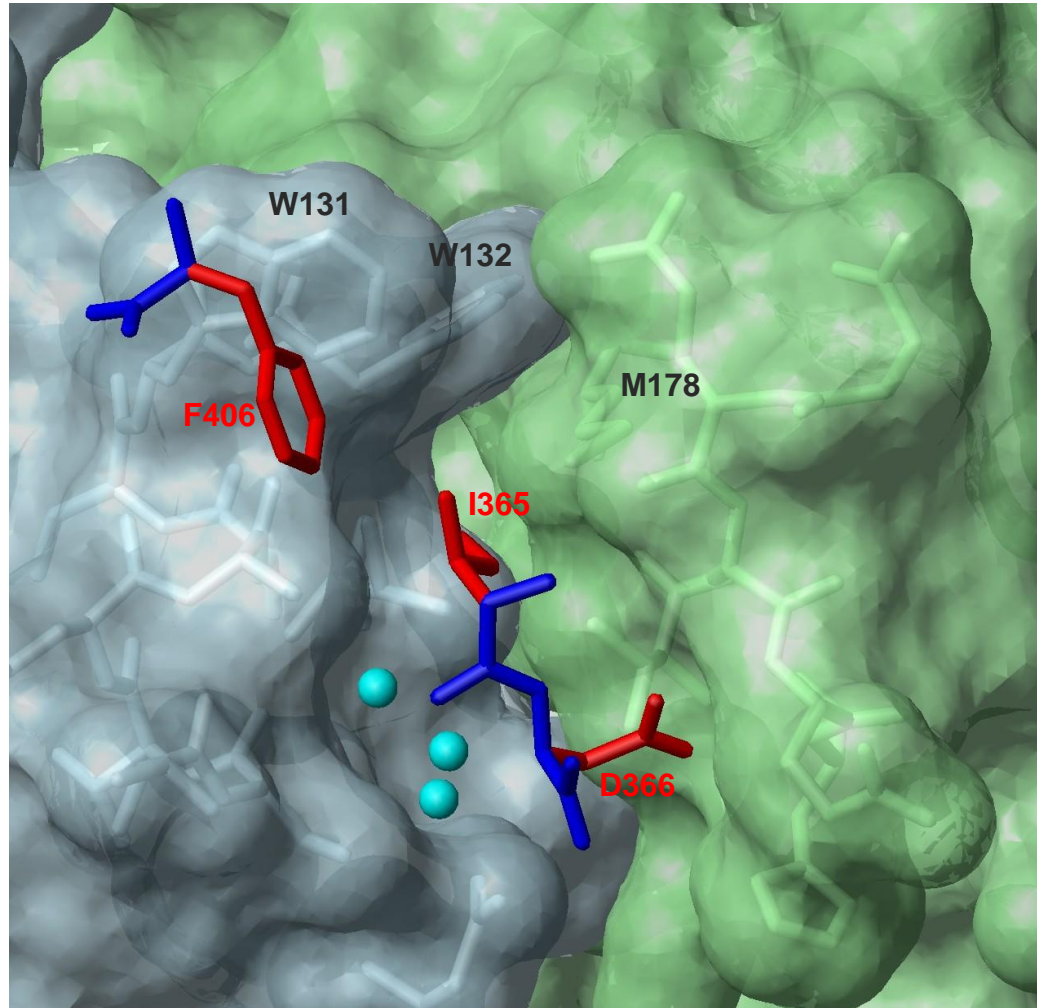
Loading of HIV PIC onto a target chromosome



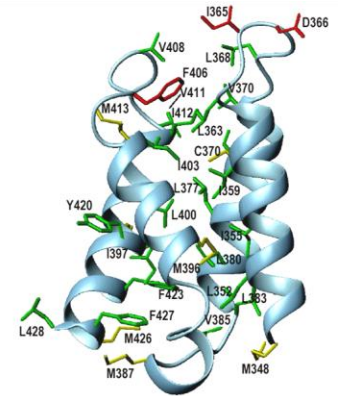
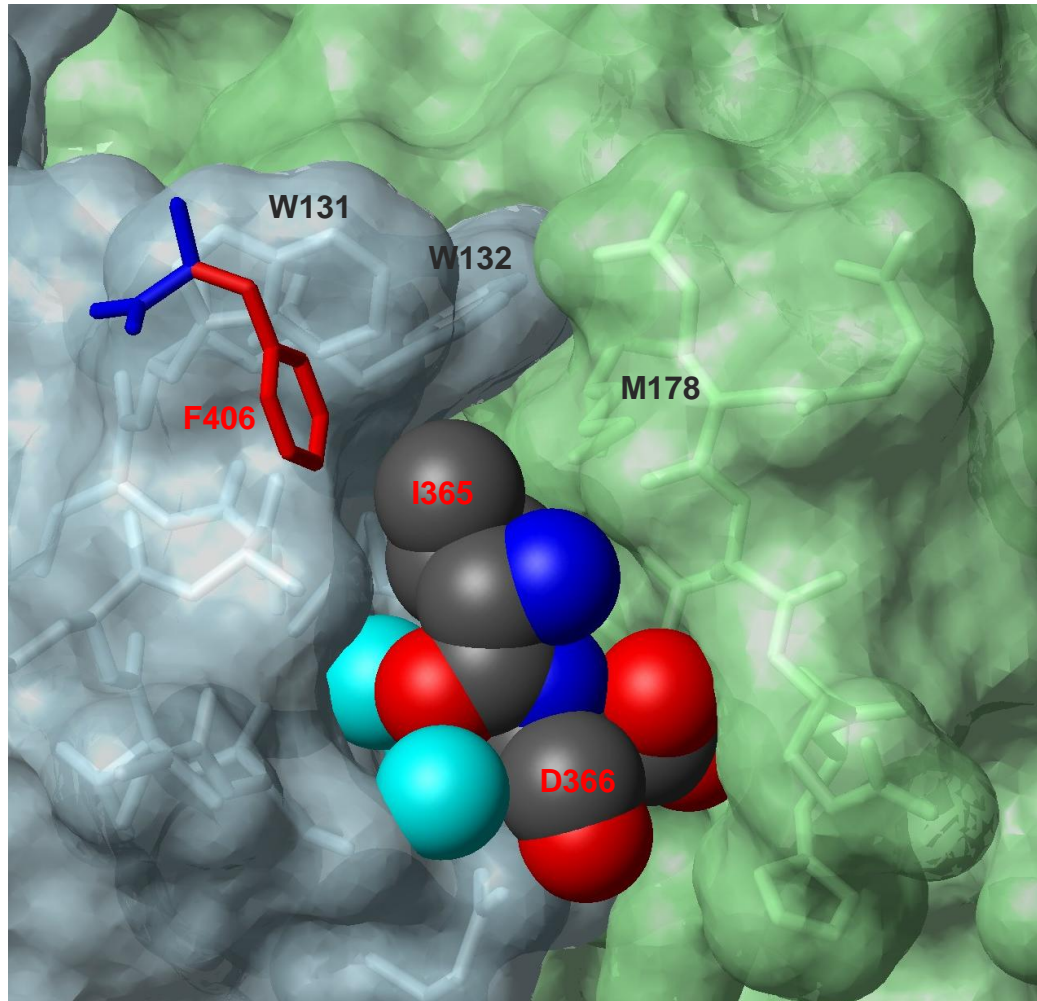
HIV-1 IN LEDGF interaction



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



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



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.
- 6) Allows for the design of inhibitors that interfere between the interaction of IN and host factors, e.g. "LEDGIns"