

Crystallographic studies on mutants of Avian Sarcoma Virus Integrase

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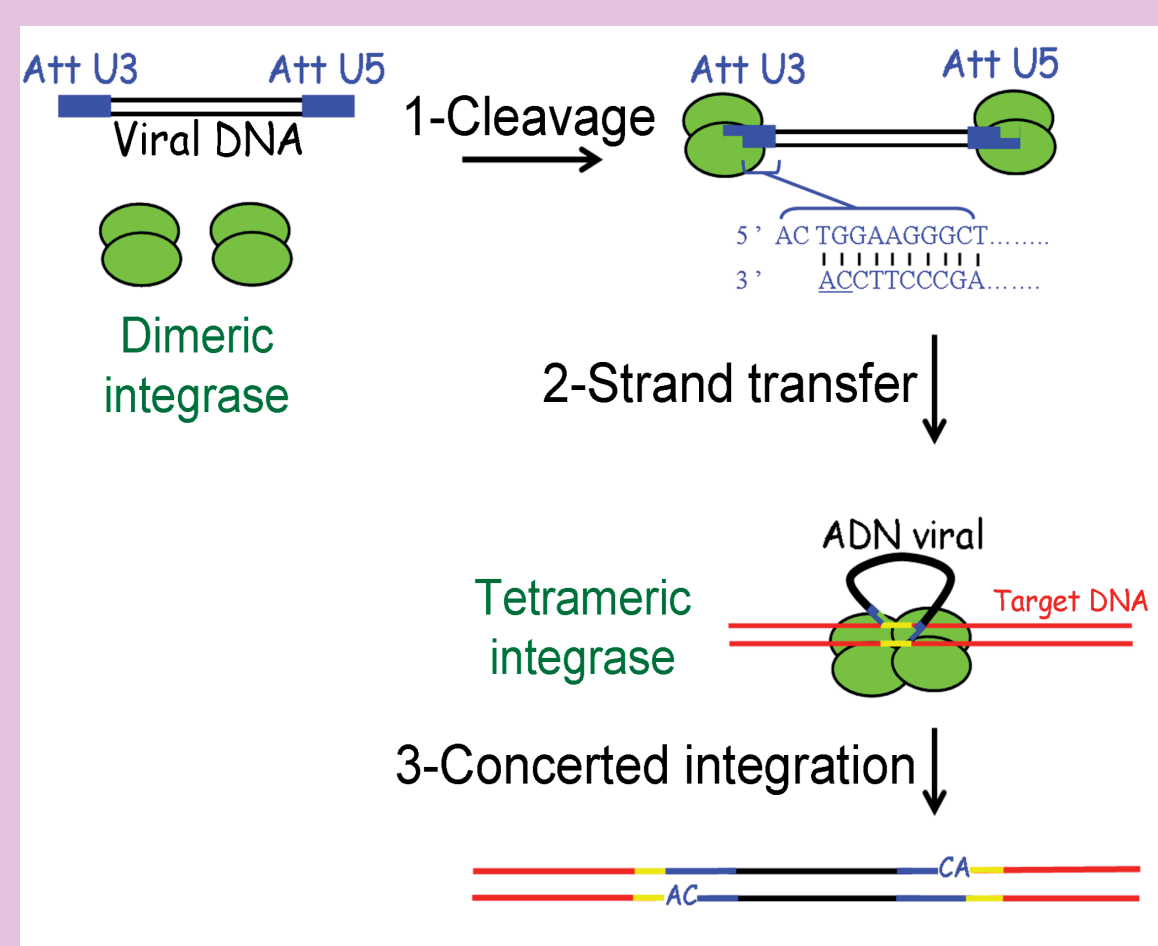
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Human immunodeficiency virus Type 1 (HIV-1) **integrase** is an essential enzyme for the obligatory **integration** of the viral DNA into the infected cell chromosome. As no cellular homologue of HIV integrase has been identified, this enzyme is an attractive **target** for the development of new therapeutics. Two integrase inhibitors are now making progress in clinical phase II trials.

The protein is made of 3 domains of known structure (**N-terminal, core, C-terminal**). These 3 domains are connected by flexible segments and the crystallographic structure of the entire protein has not been reported yet. Essential **steps of the integration mechanism** need to be clarified (i.e. conformation and oligomerization state of the protein during integration).

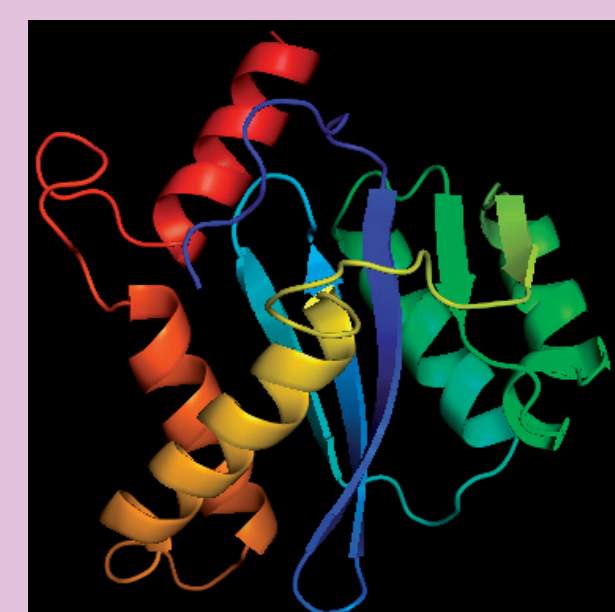
HIV-1 Integrase - HIV IN



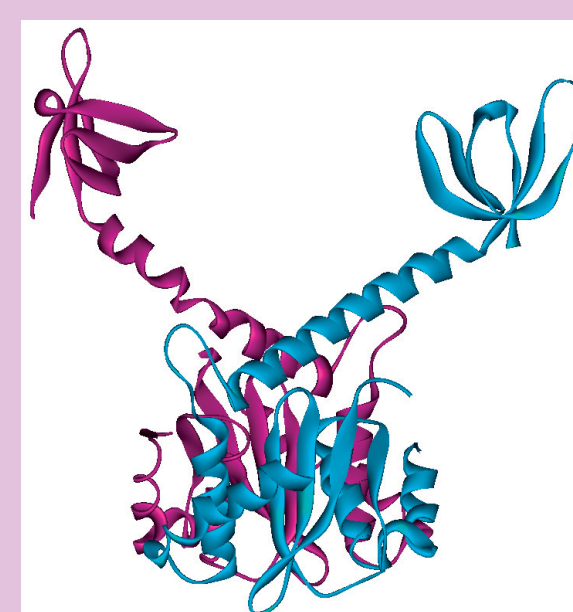
Mechanism of concerted DNA integration
Faure A. *et al.* (2005)

- Integrase functions only as multimers.
- Modular protein, precipitates → Hard to crystallise.

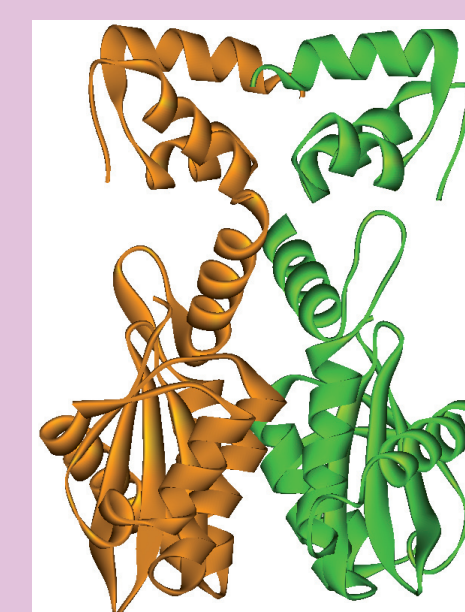
↳ Atomic structures known to date:



Core domain (1BL3)



Core + C-terminal (1EX4)



N-terminal + core (1K6Y)



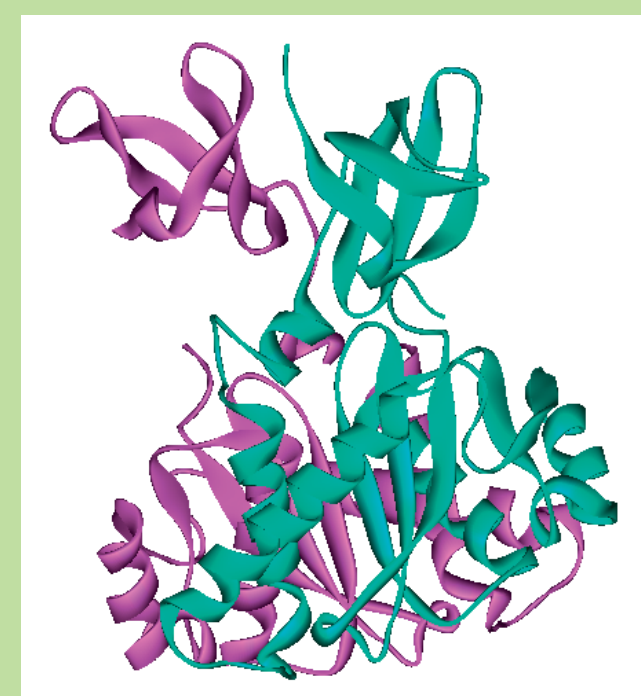
Wielens *et al.* (2005)

Three-dimensional **model** of the human immunodeficiency virus type 1 integration complex (1ZA9).

Rous Associated Virus Integrase - RAV-1 IN

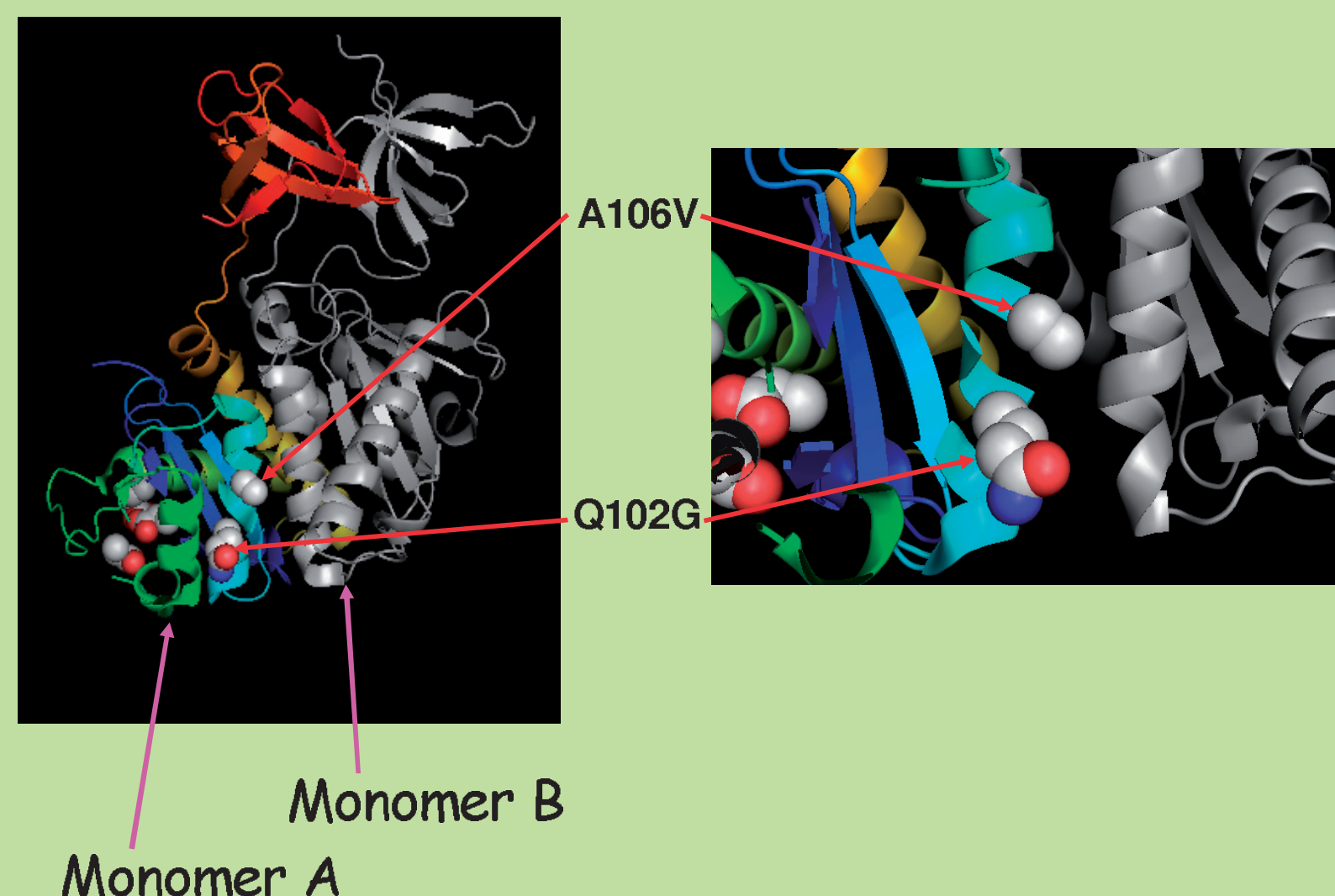
➔ Excellent model for HIV integrase:

- Same function.
- Conserved structure.
- Overexpression, purification, concentration, yield and stability are better than HIV integrase.



INRSV core + C-terminal (1C1A)
Yang Z.N. *et al.* (2000)

➔ Studies on two mutants (Q102G and A106V) located in core domain, at dimeric interface:

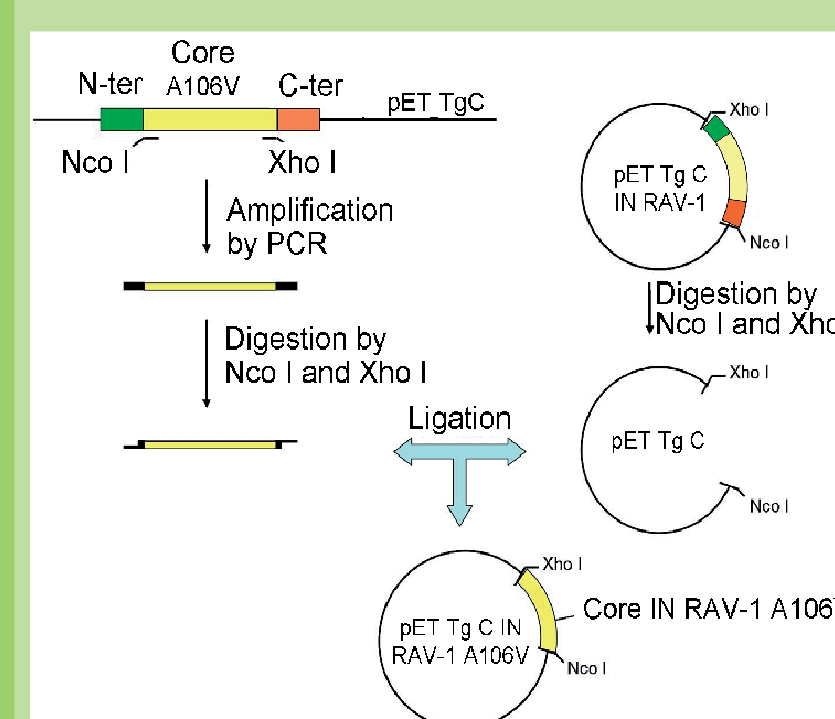


Higher biological activity than the wild-type protein.

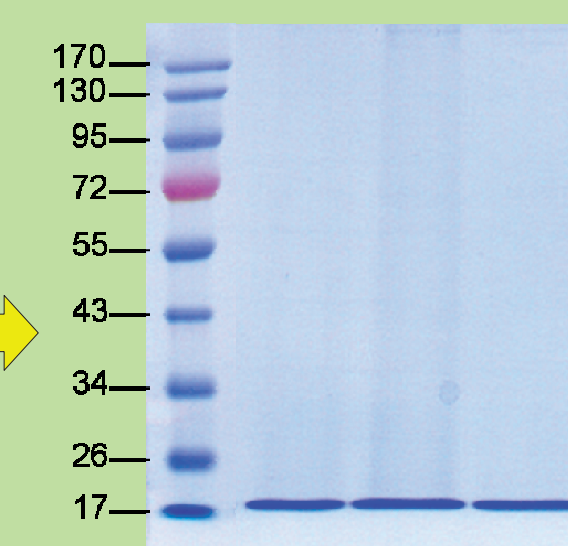
↓
Structural rearrangement?

↓
Crystallographic study.

➔ Construction of A106V mutant, overexpression, purification and crystallisation tests:



Cloning

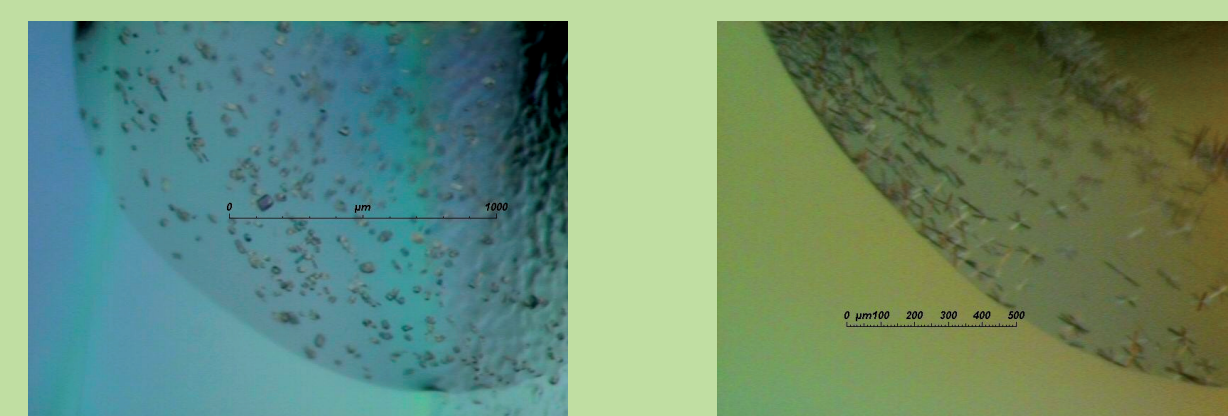


Overexpression and purification (8 mg to 13 mg/mL)



Nanodrops with Mosquito[®] robot (900 conditions tested)

➔ Getting small crystals:



Small crystals obtained with two conditions.

↓
Data collection trials at ESRF.

↓
No diffraction.

In prospect:

- **RAV integrase:**
 - Crystallographic structure of A106V mutant.
 - Overexpress, purify and crystallise Q102G mutant.
 - Co-crystallise entire protein with a target DNA.
- **HIV integrase:** integration mechanism studies with the help of 3D modelling.

References:

- Wielens J., *et al.* (2005) A three-dimensional model of the human immunodeficiency virus type 1 integration complex. *J. Comput. Aided Mol. Des.* **19**, 301-317
- Moreau K., *et al.* (2004) Mutational analyses of the core domain of Avian Leukemia and Sarcoma Viruses integrase: critical residues for concerted integration and multimerization. *Virology* **318**, 566-581
- Moreau K., *et al.* (2003) Mutations in the C-terminal domain of ALSV (Avian Leukemia and Sarcoma Viruses) integrase alter the concerted DNA integration process *in vitro*. *Eur. J. Biochem.* **270**, 4426-4438