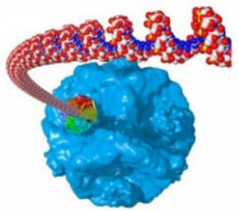
Structural snapshots of ATPase cycle of tick-borne encephalitis virus NS3 helicase



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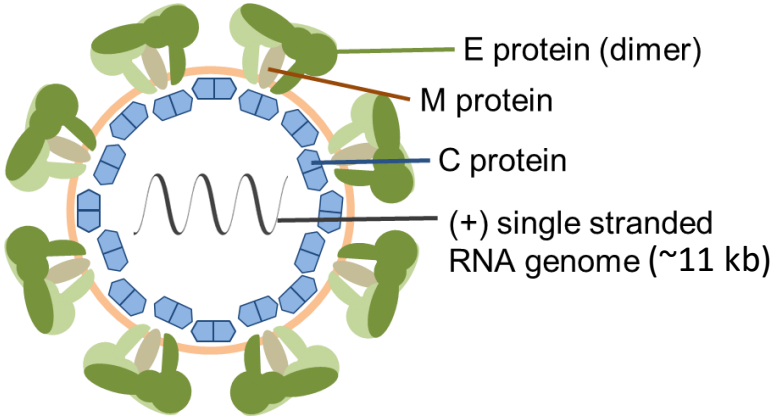
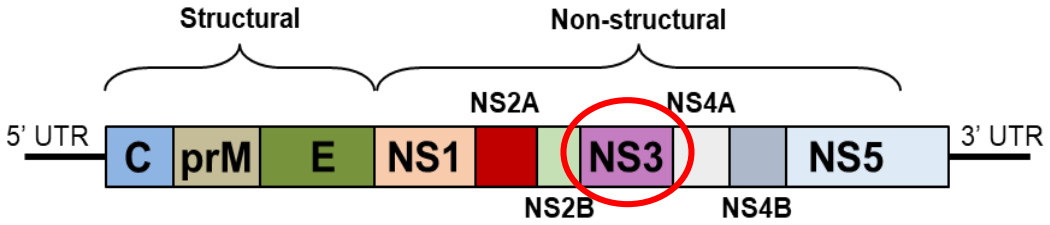
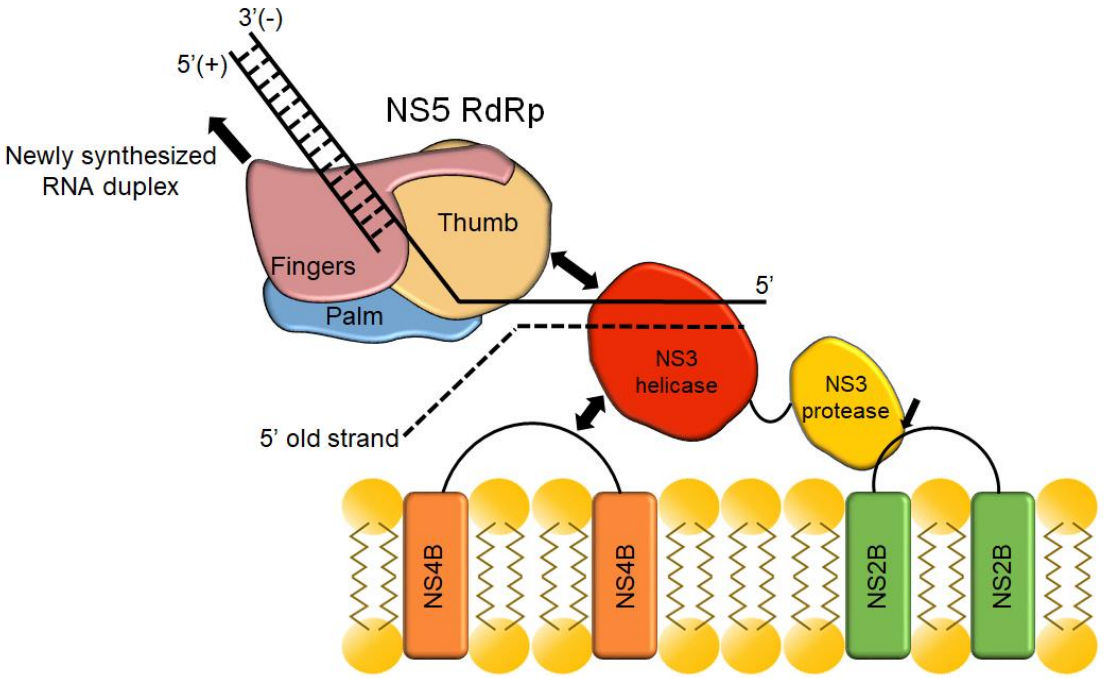
Tick-borne encephalitis virus (TBEV)
Family: *Flaviviridae;* Genus: *Flavivirus*







Modified from Luo et al., 2015

Production condition

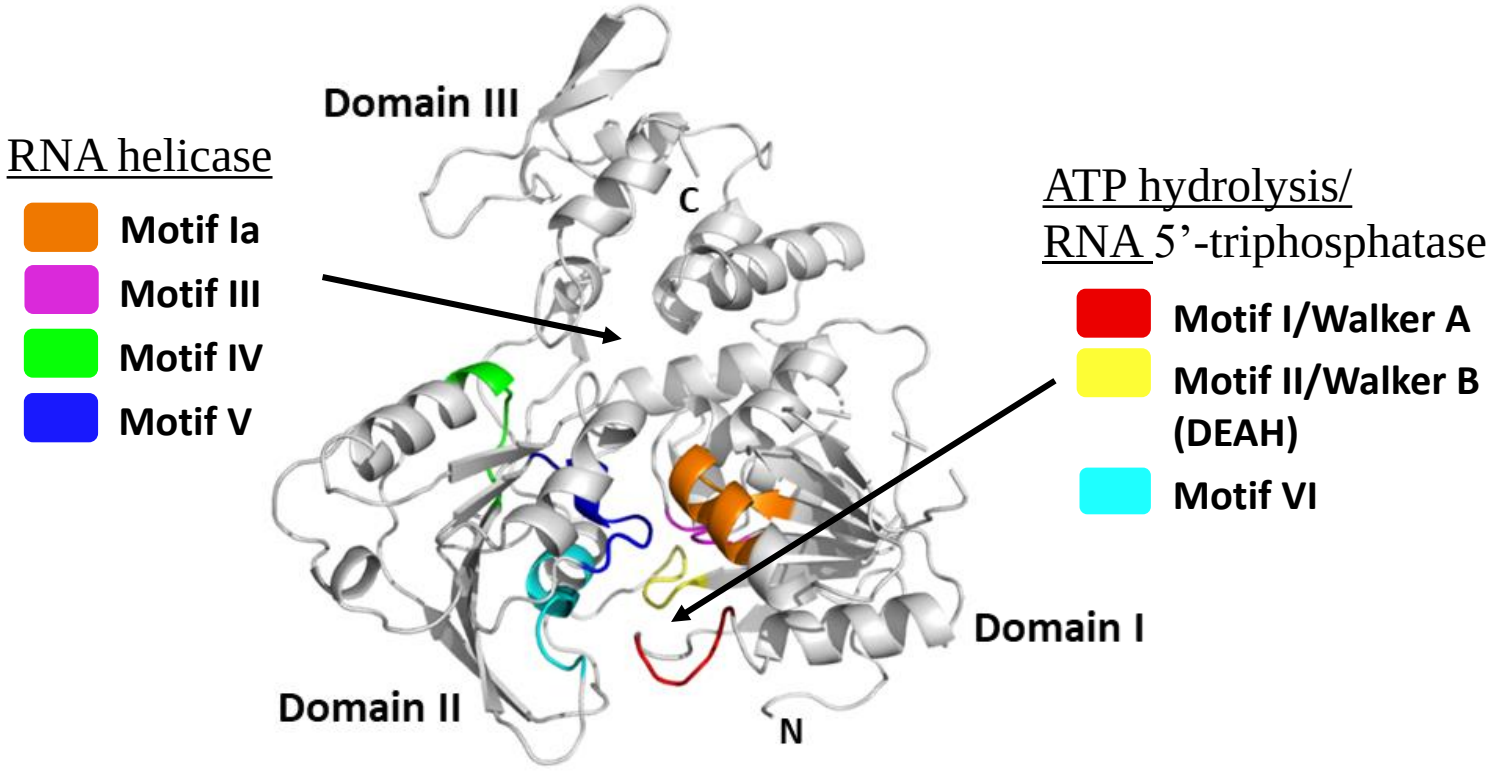
Plasmid vector	pET19b-NS3H
Competent cells	*E. Coli* BL-21 (DE)-Codonplus RIPL
Expression condition	IPTG induction, 18°C, overnight

Crystallization condition

Method	Sitting-drop vapour diffusion
Plate type	96-well plate
Temperature (K)	295
Protein concentration (mg ml^{-1})	2.2
Buffer composition of protein solution	20 mM Na-HEPES pH 7.0, 150 mM NaCl
Composition of reservoir solution	0.1 M Bis Tris Propane pH 6.5, 0.2 M Sodium acetate trihydrate, 20% w/v PEG 3350, 10% v/v Ethylene glycol
Volume and ratio of drop	1 µl, 1:1 and 1:0.5
Volume pf reservoir (µl)	50

Data collection

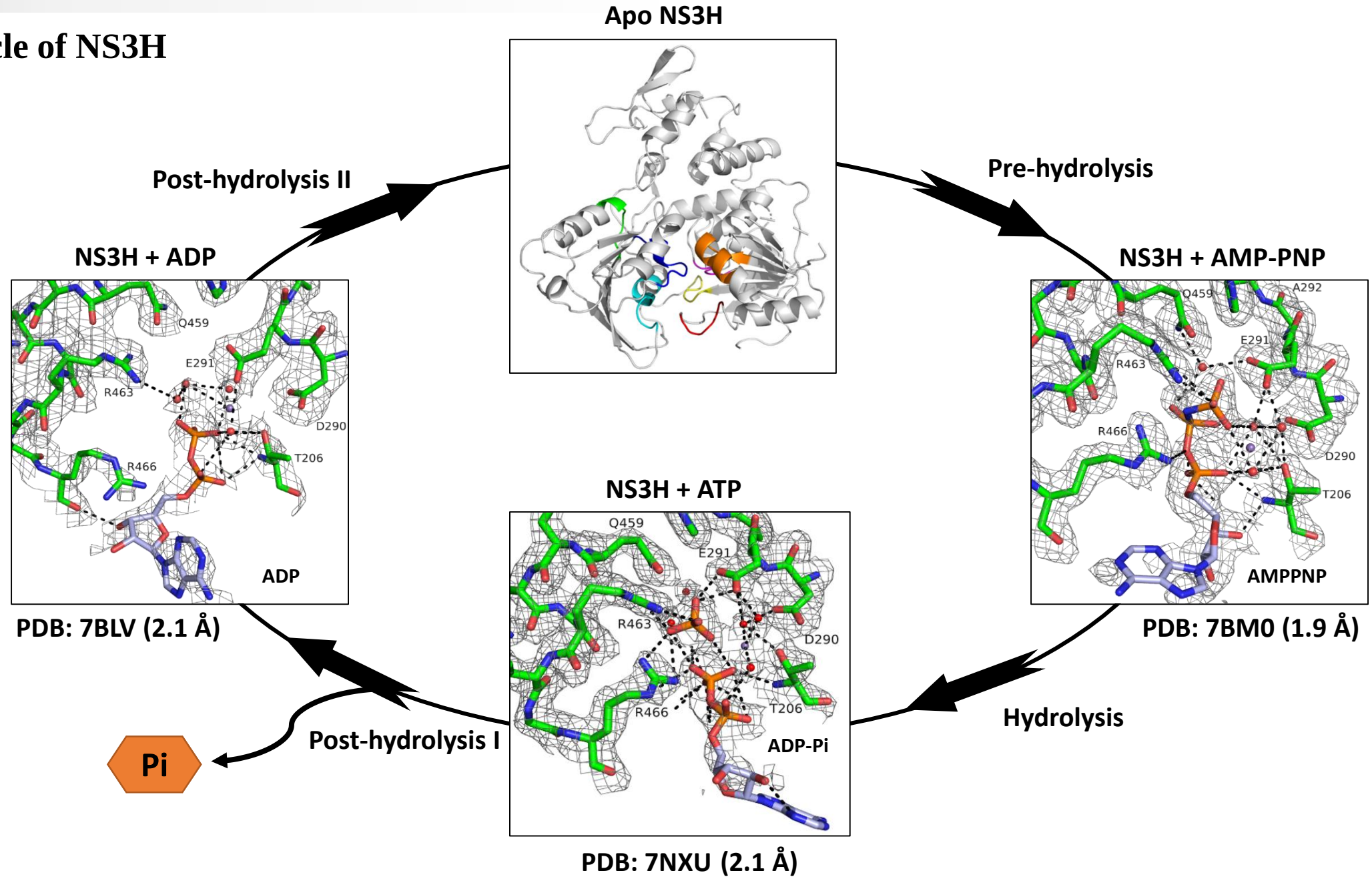
Diffraction source	BL14.1, BESSY II
Wavelength (Å)	0.9184
Temperature (K)	100
Detector	PILATUS 6M

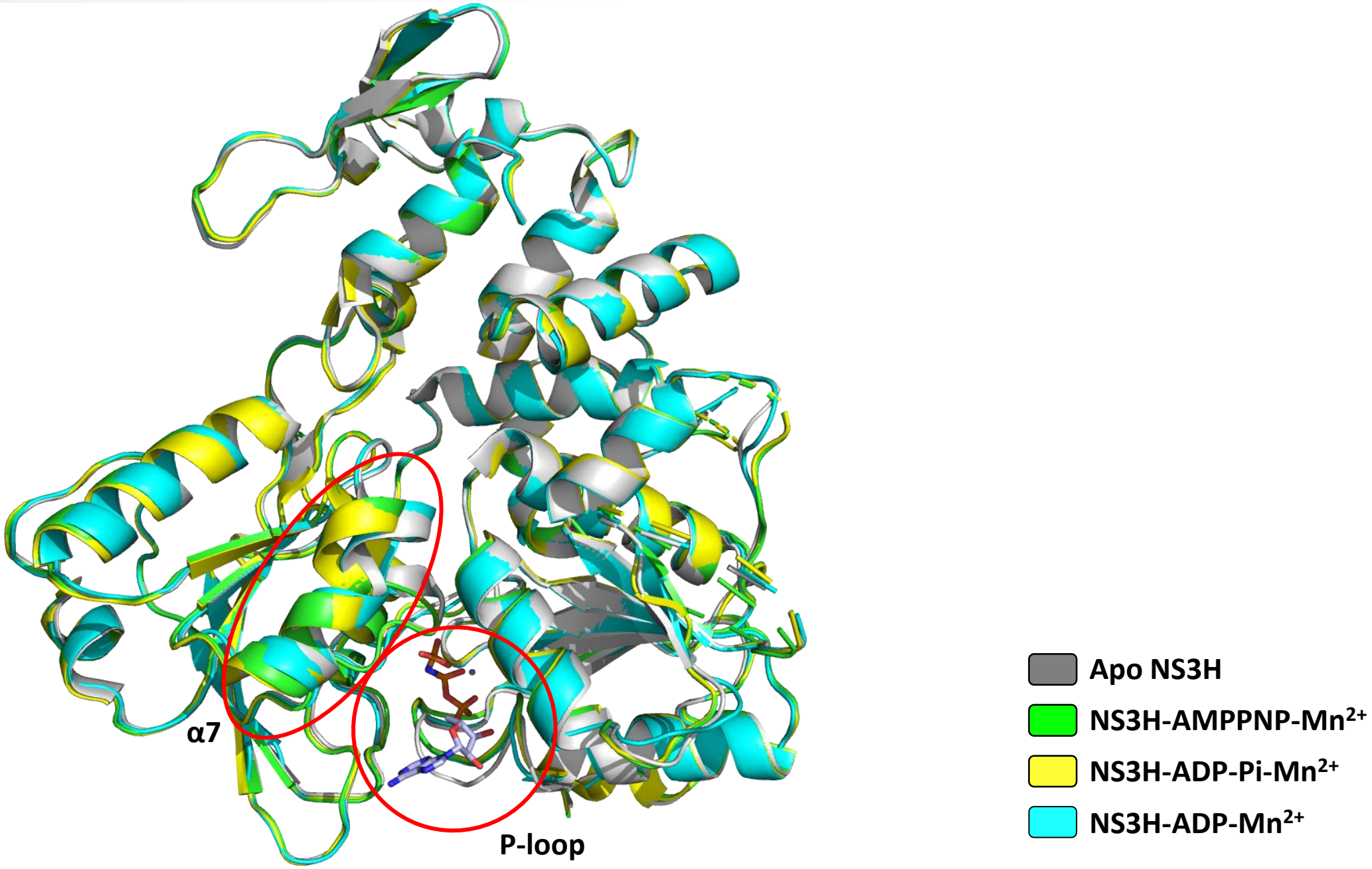


Apo NS3 helicase, PDB: 7AY4 (1.83 Å)

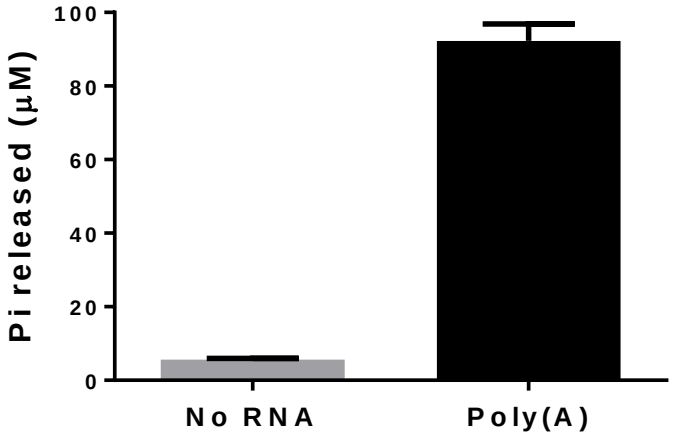
Structural snapshots of ATPase cycle of TBEV NS3 helicase
Anindita et al.

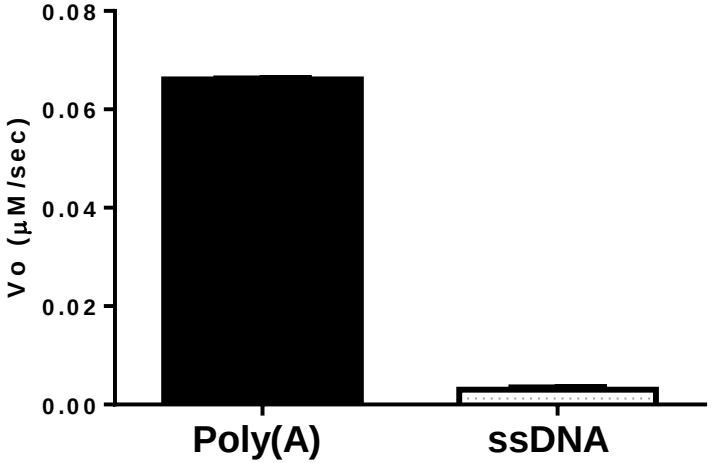
ATPase cycle of NS3H



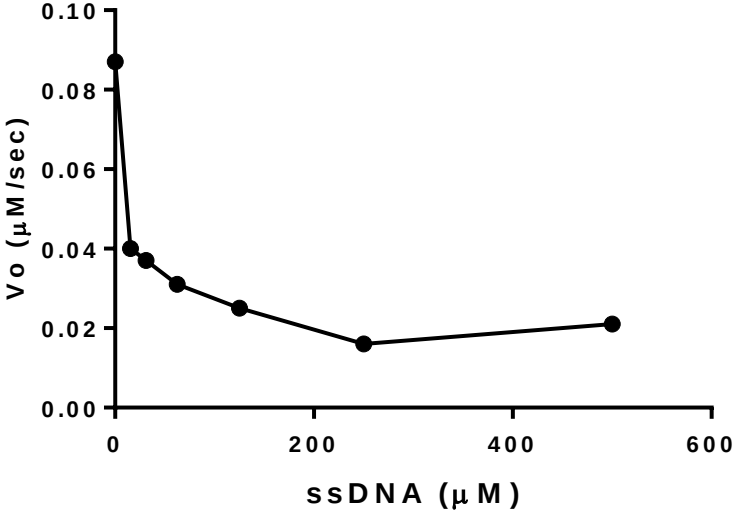

α7
P-loop
Apo NS3H
NS3H-AMPPNP-Mn2+
NS3H-ADP-Pi-Mn2+
NS3H-ADP-Mn2+

Presence of ssRNA better stimulates inorganic phosphate (Pi) release than ssDNA.





ssDNA could bind competitively to the nucleic binding site against poly(A).



Acknowledgement

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- For molecular dynamic (MD) simulation of NS3H-RNA-ATP binding and Pi release pathway, please visit poster presentation by Marco Halbeisen (Poster Session 2-B).