Mechanism of core protein allosteric modulators (CpAMs) Misdirection of Hepatitis B Virus Capsid Assembly

Shitian Wei (Class of 2020), Ju-Tao Guo (Supervisor), John Kulp (Second Reader), Ana-Rita Mayol (Advisor)
Baruch S. Blumberg Institute (BSBI), Lab 257, 3805 Old Easton Road, Doylestown PA 18902, USA

Introduction

- Hepatitis B virus (HBV) chronically infects more than 250 million people worldwide and causes 680, 000 deaths annually.
- **Capsid formation** plays an important role in HBV life cycle. Inhibition of assembly provides a new direction in development of novel antiviral agents against chronic HBV infection.
- HBV **core protein allosteric modulators (CpAMs)** disrupt HBV capsid assembly by interacting with the hydrophobic interface of **core protein (Cp)** dimers, the building block of HBV capsid.
- Confounding effects in cell and bacteria environment influence capsid assembly and hinder research on mechanism of CpAM misdirection.
- This project intended to establish an *in vivo* capsid assembly assay, to further understand the mechanism of HBV capsid assembly through interaction between CpAMs and Cp dimer.

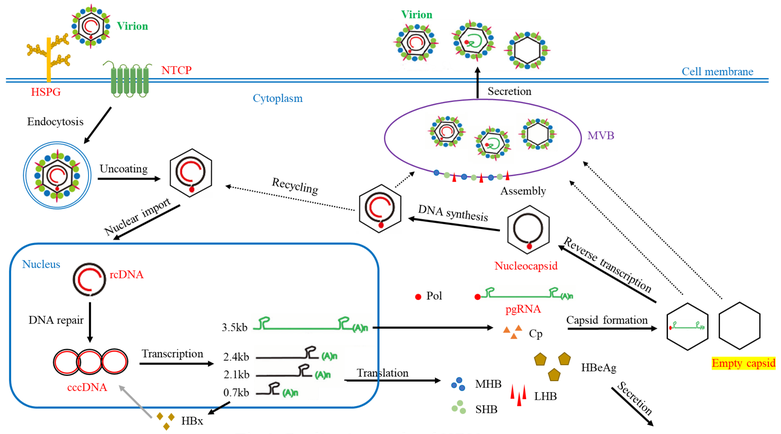


Fig 1. Replication cycle of HBV.

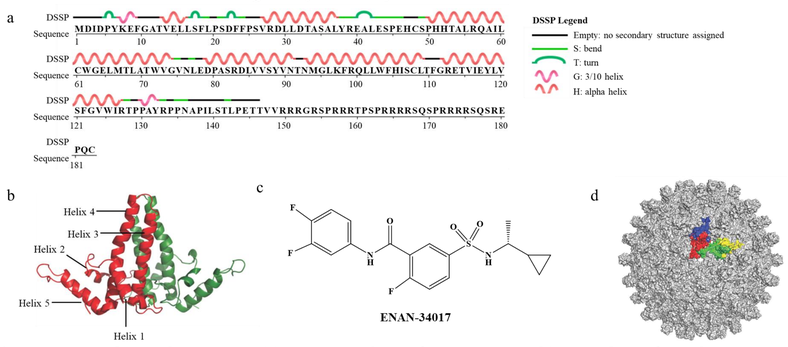
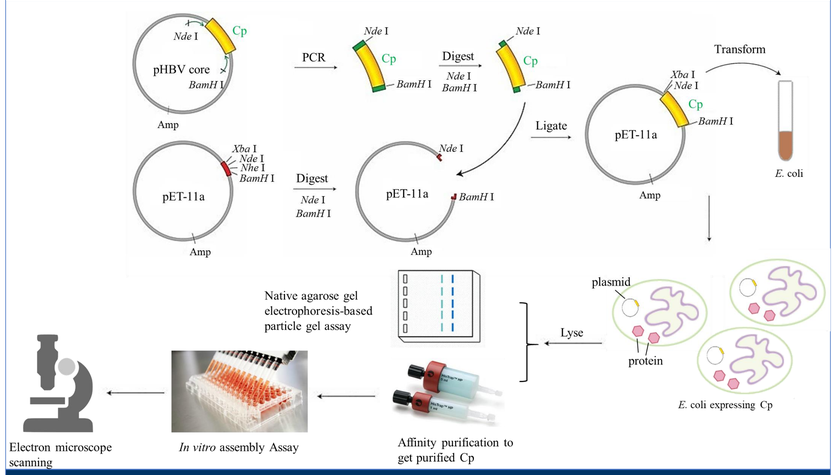


Fig 2. Dimer and capsid structures. (a) Sequence chain of core protein. (b) A Cp dimer from the context of a capsid. (c) Structure of ENAN-34017. (d) Cp T = 4 capsid with the asymmetric unit in color. The individual subunits are A (blue), B (red), C (green), and D (yellow) or AB and CD dimers.

References

1. Wizemann, H.; Von Brunn, A. Purification of E. coli-expressed HIS-tagged Hepatitis B Core Antigen by Ni^{2+}-chelate Affinity Chromatography. *J. Virol. Methods.* **1999**, *77* (2), 189-197.
2. Wu, S.; Zhao, Q.; Zhang, P.; Kulp, J.; Hu, L.; Hwang, N.; Zhang, J.; Block, T. M.; Xu, X.; Du, Y. Discovery and Mechanistic Study of Benzamide Derivatives that Modulate Hepatitis B Virus Capsid Assembly. *J. Virol.* **2017**, *91* (16), e00519-17.

Experimental Design



Results

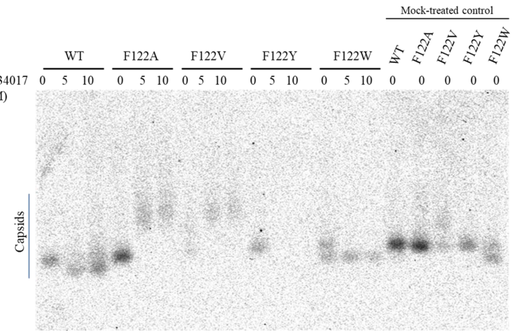


Fig 3. Particle gel assay of capsids assembled in HepG2 cells treated with ENAN34017.

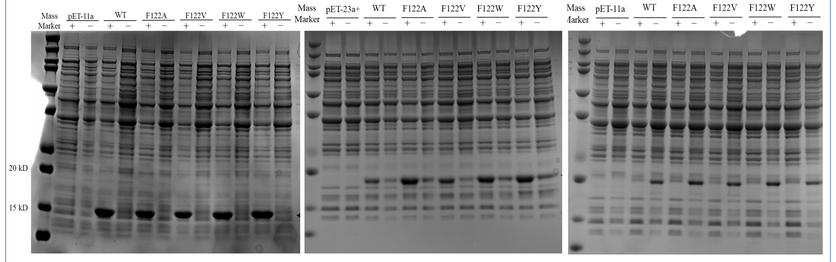


Fig 4. Validation of $Cp^{1\text{-}149}$, C-terminally His-tagged $Cp^{1\text{-}149}$, $Cp^{1\text{-}183}$ protein expression by SDS-PAGE gel.

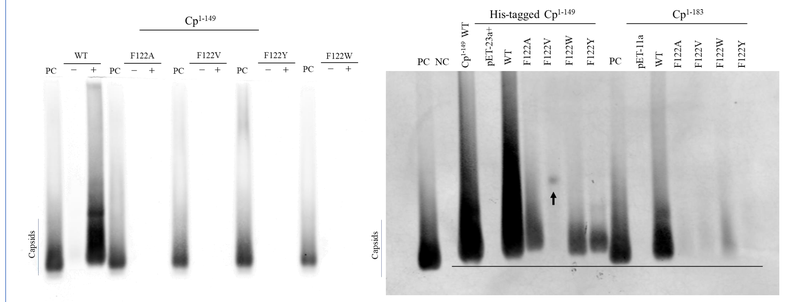


Fig 5. Validation of $Cp^{1\text{-}149}$, C-terminally His-tagged $Cp^{1\text{-}149}$ and $Cp^{1\text{-}183}$ protein capsid assembly by particle gel assay(PC: YFB cell lysate from Zhanying, NC: 1mg/mL lysozyme).

Results

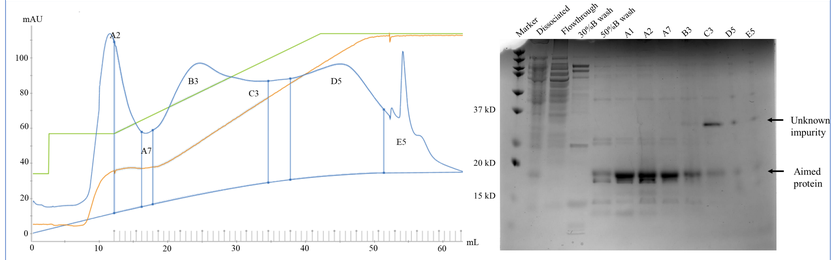


Fig 4. Purification curve of C-terminally His-tagged $Cp^{1\text{-}149}$ WT from ÄKTA pure Protein Purification System and validation of purification by SDS-PAGE gel.

- Large quantity of protein was unable to bind His-Trap column without dissociation of capsid and got lost in flow through

Conclusion

- Overexpression system was successfully established for $Cp^{1\text{-}149}$, $Cp^{1\text{-}183}$, and C-terminally His-tagged $Cp^{1\text{-}149}$ WT and four mutants by *E. coli*.
- Mutants showed unstable assembly in E. coli, but relatively stable assembly in mammalian cells. Capsid assembly in different cell environments are different, which emphasize the necessity to dismiss confounding effects.
- C-terminally His-tagged $Cp^{1\text{-}149}$ WT was successfully purified through affinity column chromatography.

Future Plan

- Further purification through size-exclusion column and ion-exchange column will be applied to C-terminally His-tagged $Cp^{1\text{-}149}$.
- An *in vivo* capsid assembly/disassembly assay will be established through adjustment of salt concentration and pH.
- Key biophysical parameters, including size, assembly/disassembly kinetics, and thermal stability w/o CpAM treatment of assembled capsids will be obtained under electron microscope (EM) scanning.

Acknowledgement

Professor
Ju-Tao Guo, MD
Jinhong Chang, MD, PhD

Group members
Haiqun Ban
Zhanying Hu
Sisi Yang
Qiong Zhao
Hui Liu
Zhao Gao
Lin Zhang
Julia Ma