Dual Mechanism of Actions of Novel HBV Core Protein Allosteric Modifiers (CpAMs): Inhibiting Viral Replication and Blocking cccDNA Formation

Qi Huang[1], Dawei Cai[1], Pao-Chen Li[1], Alex Mercier[1], Renuka Kumar[1], Emily Connelly[1], Yuhua(Sara) Zong[1], Ran Yan[1], Xiulan Zhou[1], Yi Zhou[1], Lida Guo[1], Ariel Tang[1], Geoffrey Chen[1], Esteban Carabajal[1], Katherine Nabel[1], Lichun Li[1], Steve Dunkelbarger[1], Sarah Katen[1], Jason Deer[1], Earl May[1], Uri Lopatin[1], Adam Zlotnick[1,2] and Richard Colonno[1]

Assembly Biosciences[1]
Indiana University[2]

assembly biosciences

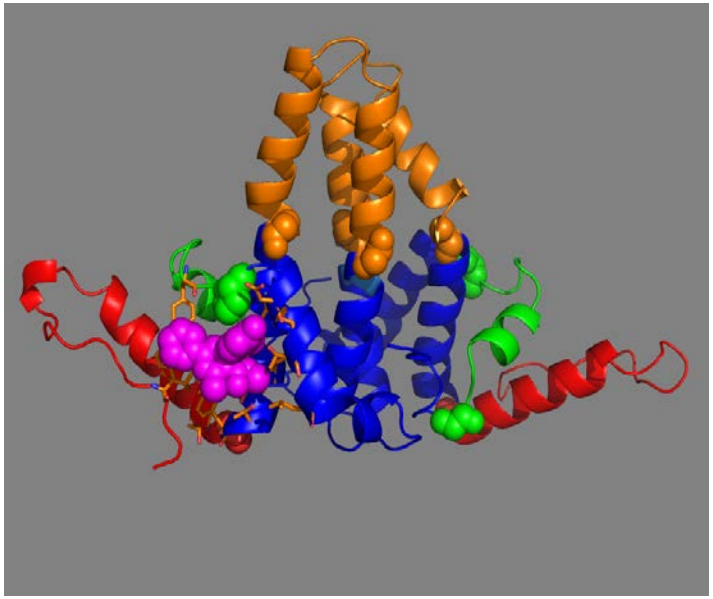
Core Protein Allosteric Modifiers (CpAMs)

Structurally distinct small molecules allosterically bind to a pocket of Core Protein at the dimer-dimer interface

Accelerate capsid assembly and induce Cp to form different sizes of aberrant capsids

Block pgRNA encapsidation and lead to the formation of empty capsids

First generation of CpAMs is in early clinical development

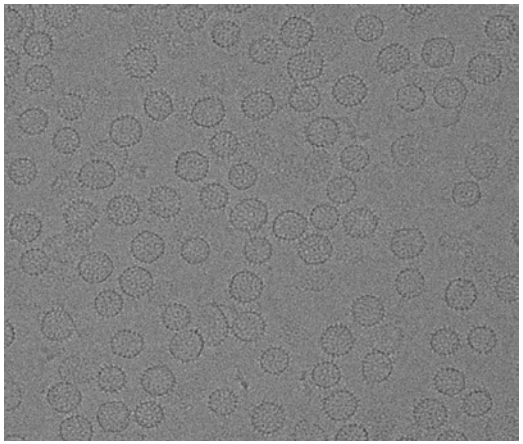


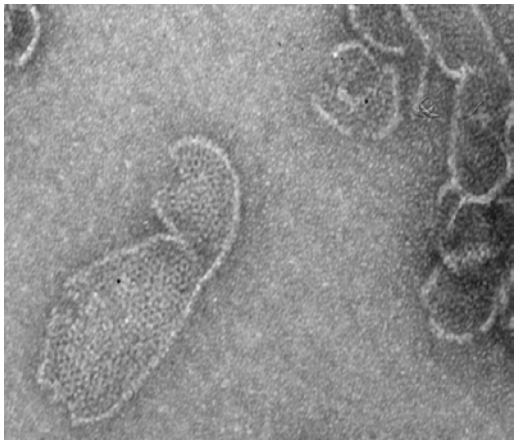
Zlotnick et al (1999) Biochemistry 38, 14644-52
Zlotnick et al (2011) Trends in Microbiology 19, 14-23

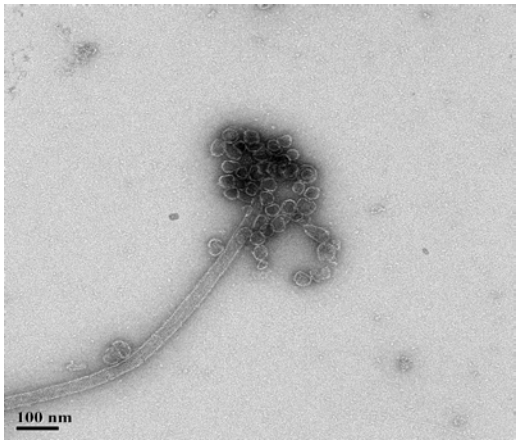
CpAMs Induce Aberrant Capsid and Cause Cp Aggregation

Normal Symmetric Capsid

Variety of irregular sized Capsid





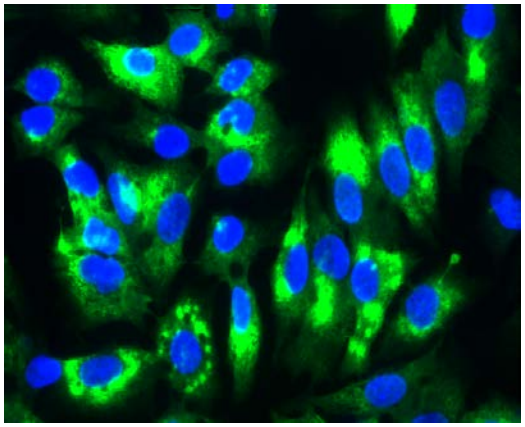


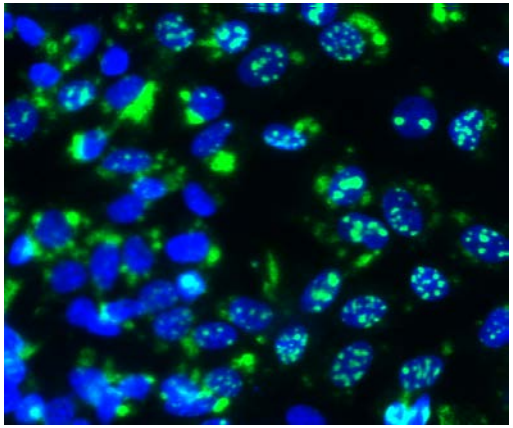
TEM
Cp150/
Capsid

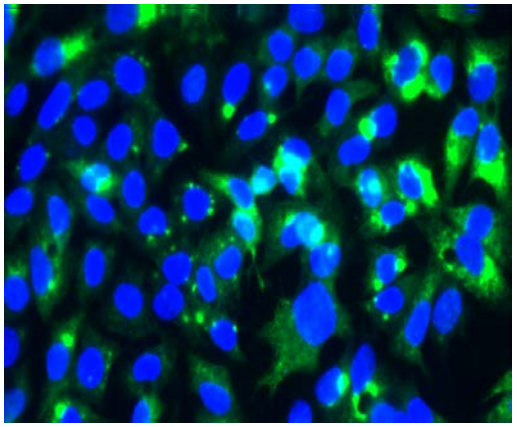
DMSO

HAPs

ABI-H0731





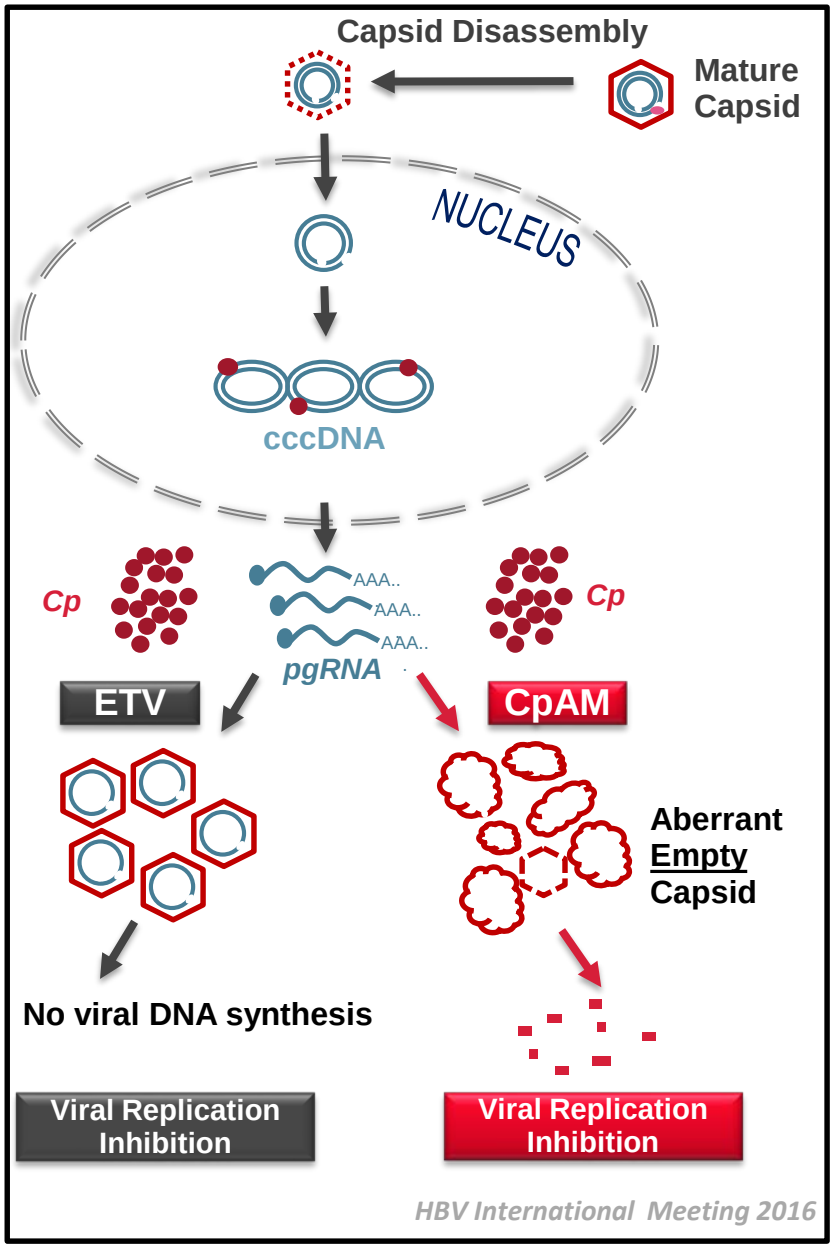


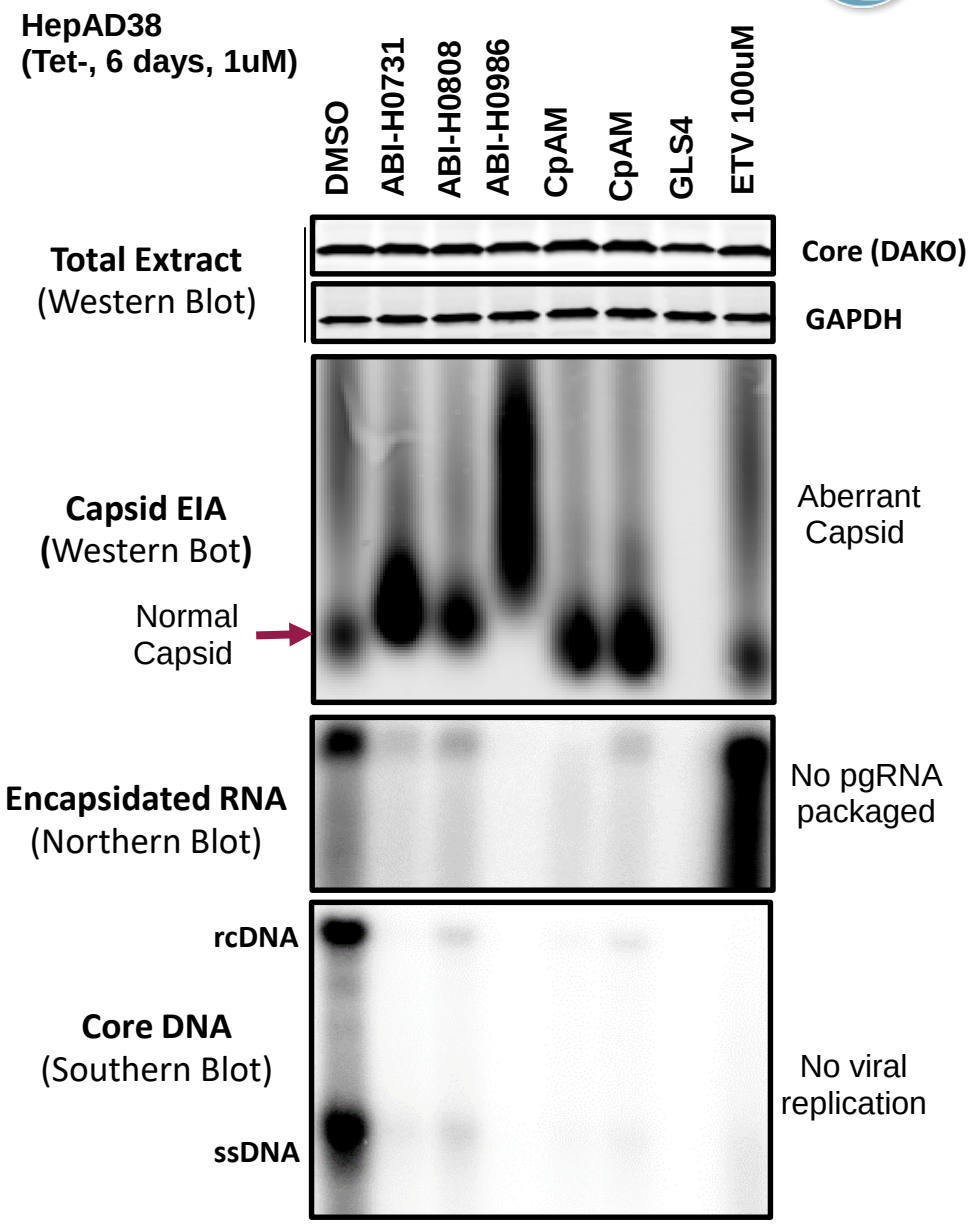
AD38 IFA
Green:
Cp/Capsid
Blue:
Nucleus

Mostly diffused cytoplasmic
Cp staining

Cytoplasmic and nuclear
aggregates

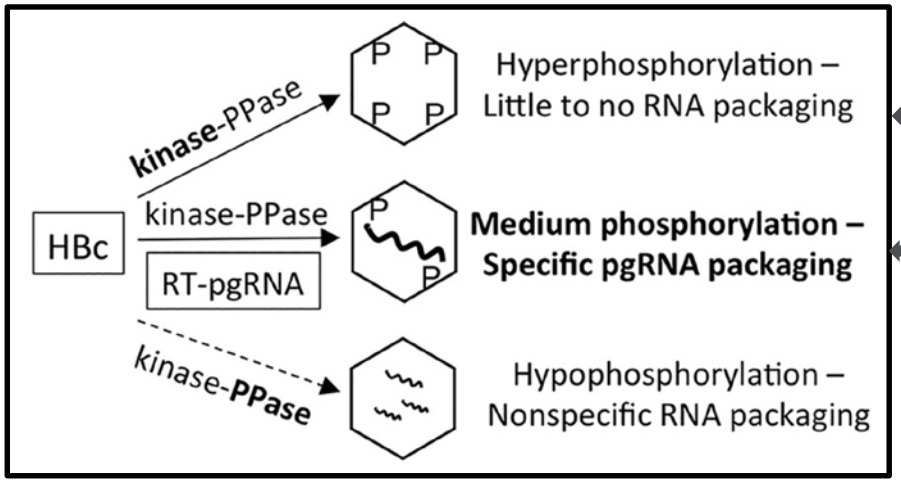
CpAMs Inhibit HBV Replication by Inducing Empty Capsid Formation





CpAMs Modify Cp Phosphorylation Status

Regulation of specific versus nonspecific RNA packaging of HBV capsids by the degree of CTD phosphorylation and dephosphorylation

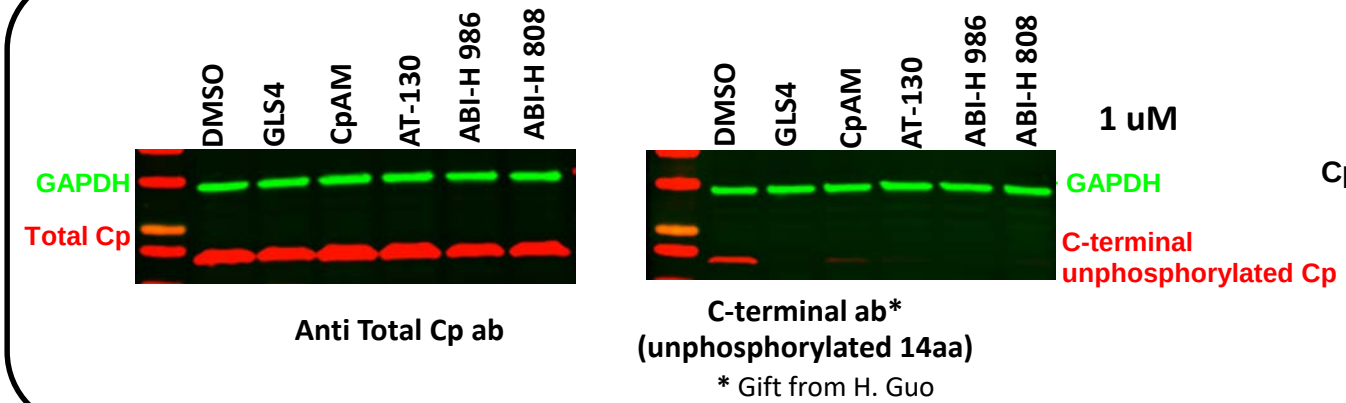


CpAMs Mimic Effect?

Normal Capsid

Hu et al (2016) J Virol 90:5830 –5844

Western Blot Evaluation of Cp Phosphorylation



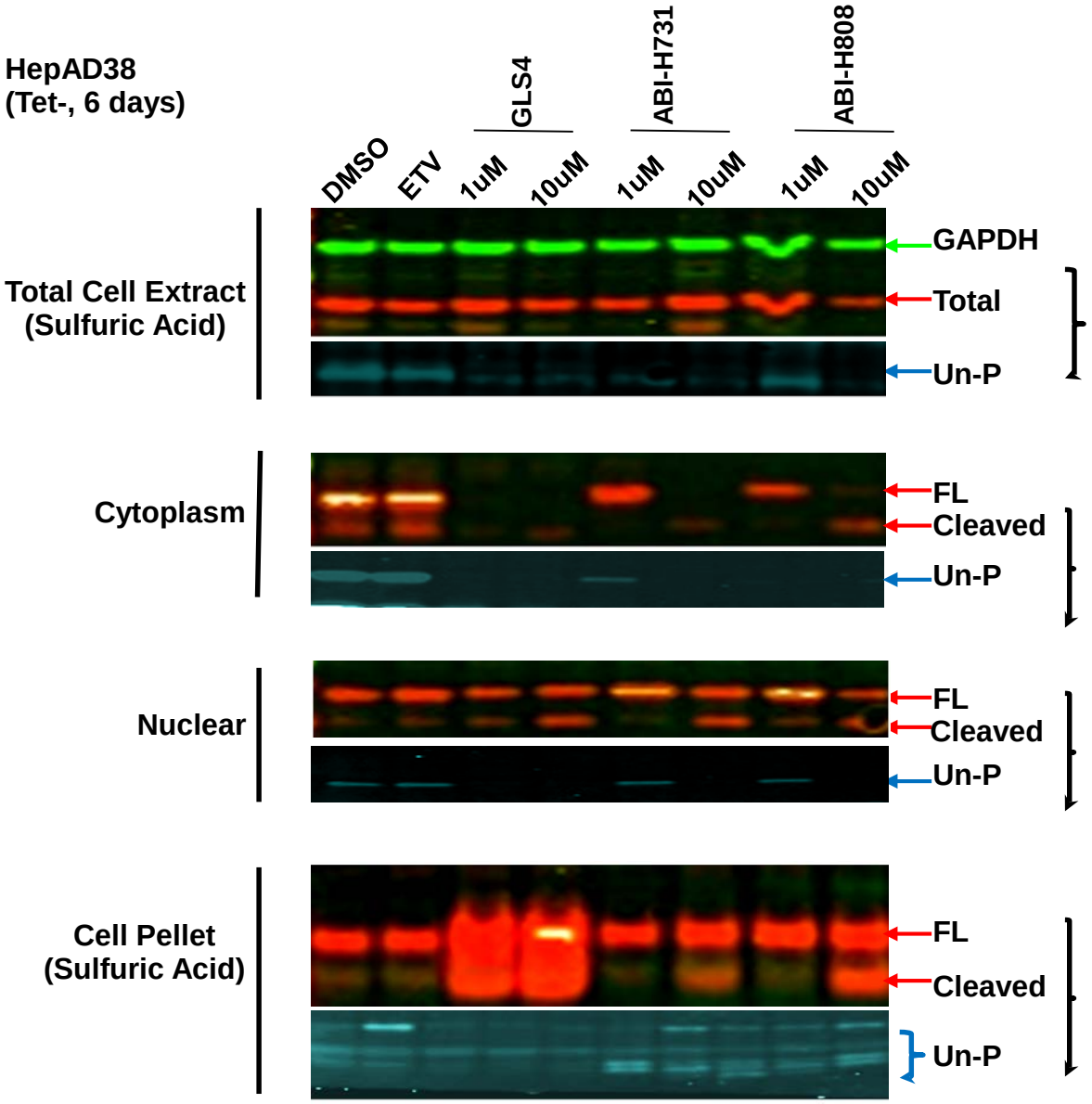
* Gift from H. Guo

2 Day treatment

No effect on Cp expression

Cp remains hyperphosphorylated

CpAMs Induce Hyperphosphorylated Cp to Aggregate and Degrade

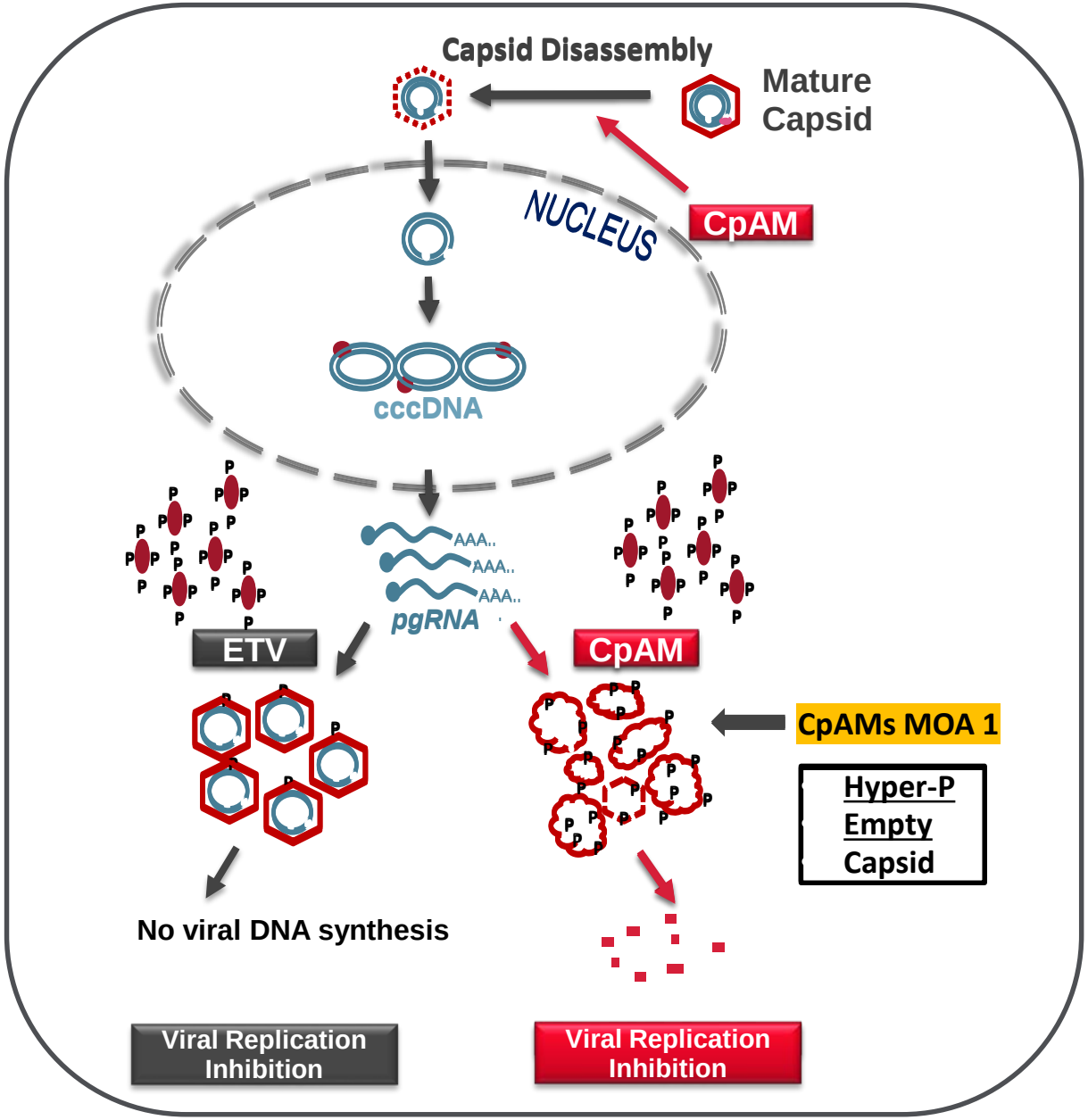


CpAM effect on Cp

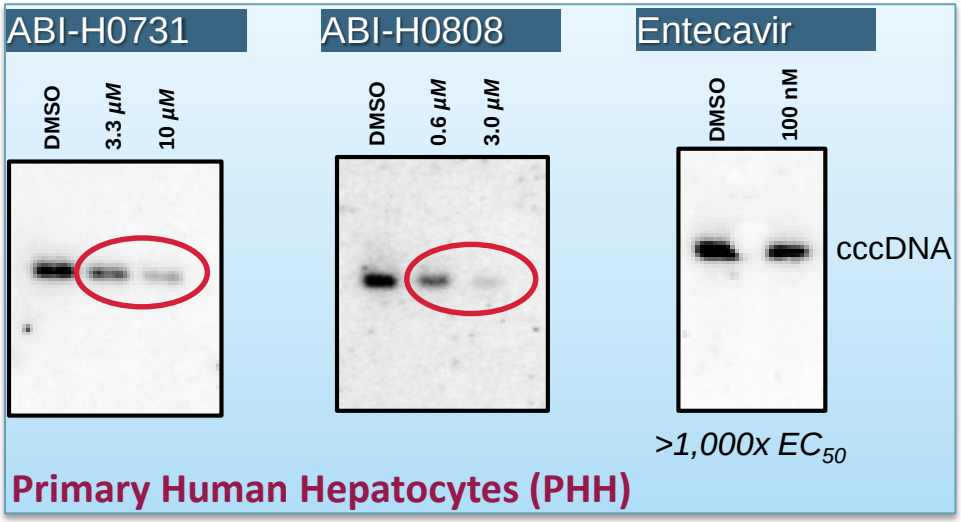
Stable total expression
Hyper-phosphorylation
Enhanced Degradation

Cleavage/Aggregation
Hyper-phosphorylation

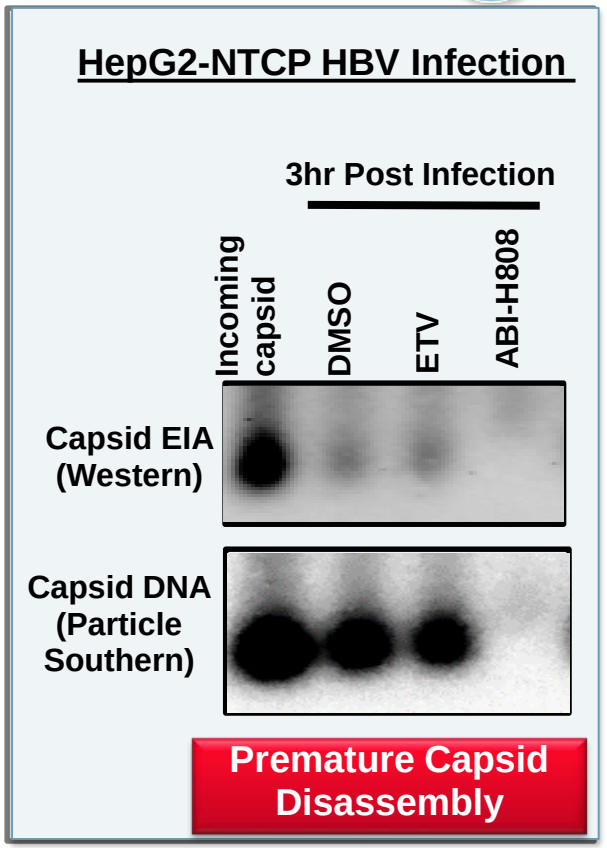
CpAMs Effectively Inhibit HBV Viral Replication

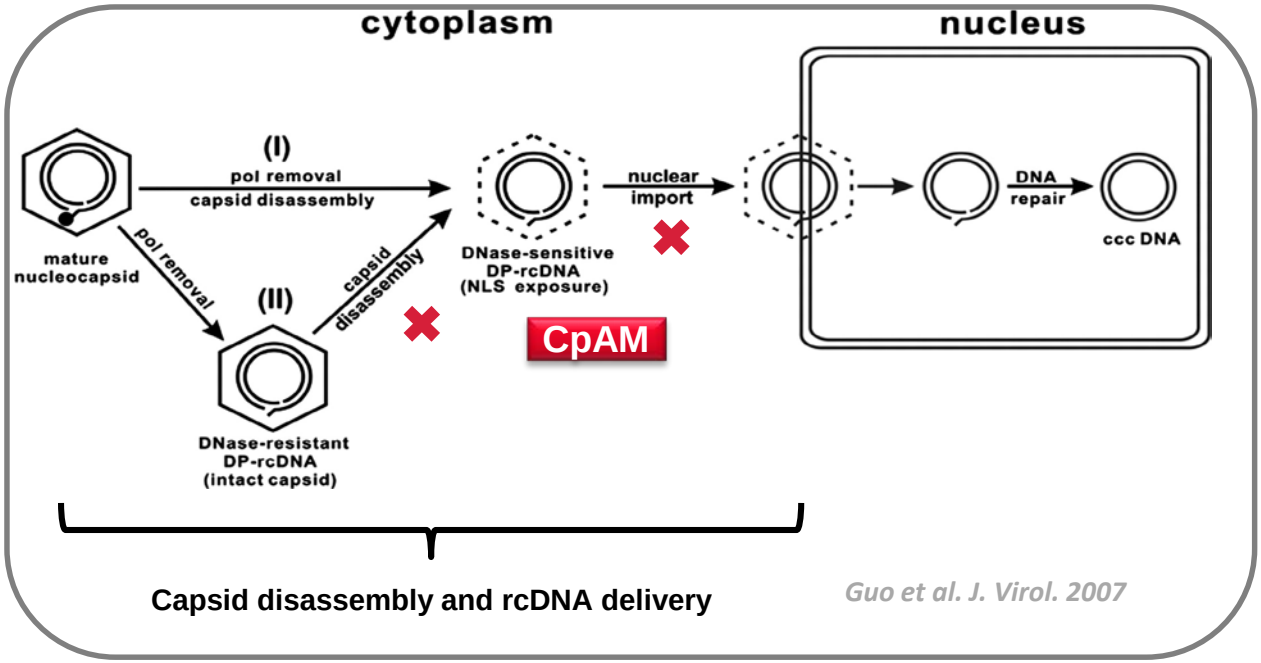


CpAMs block cccDNA Formation in HBV Infected Cells

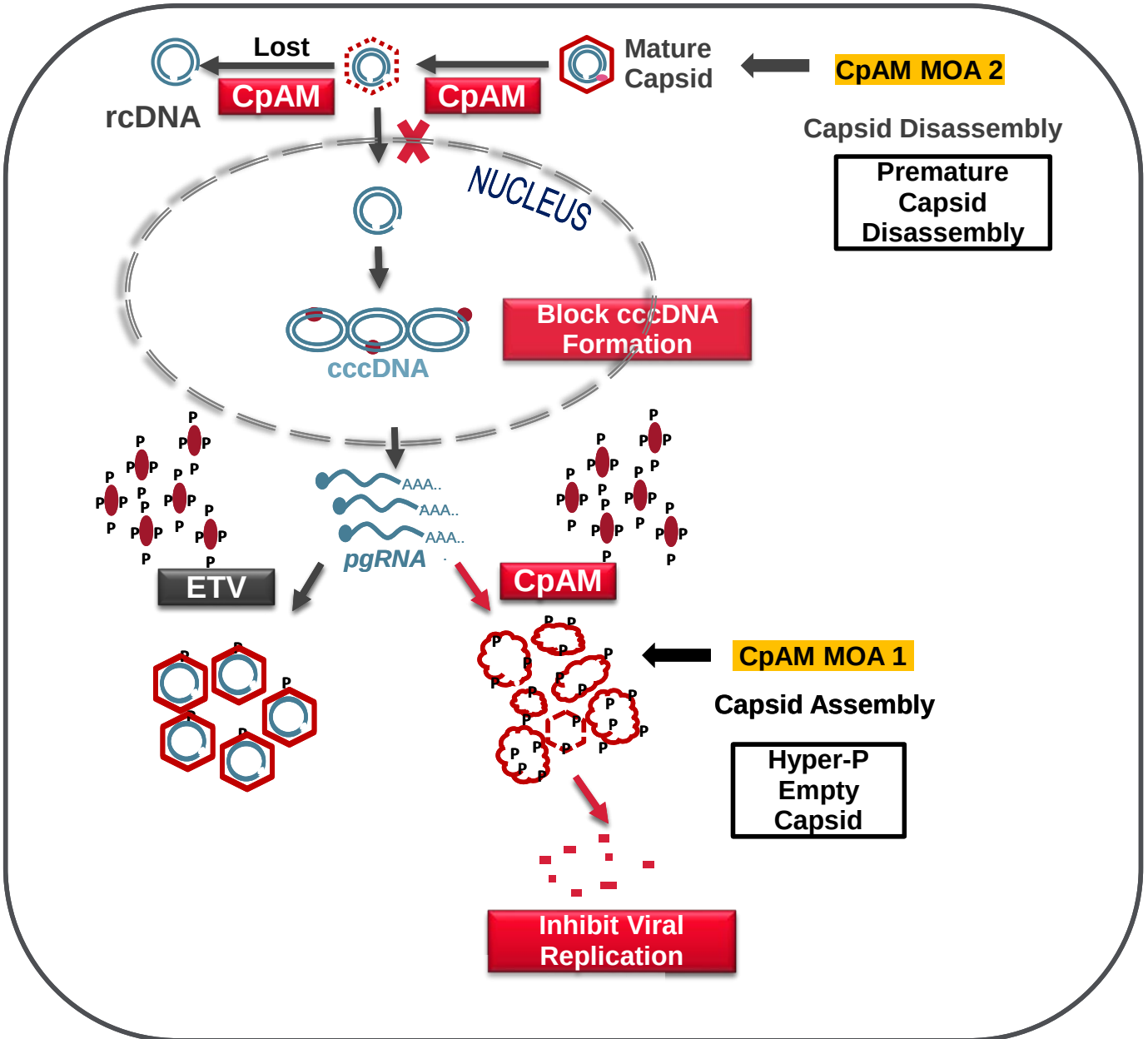


Presented at AASLD 2016

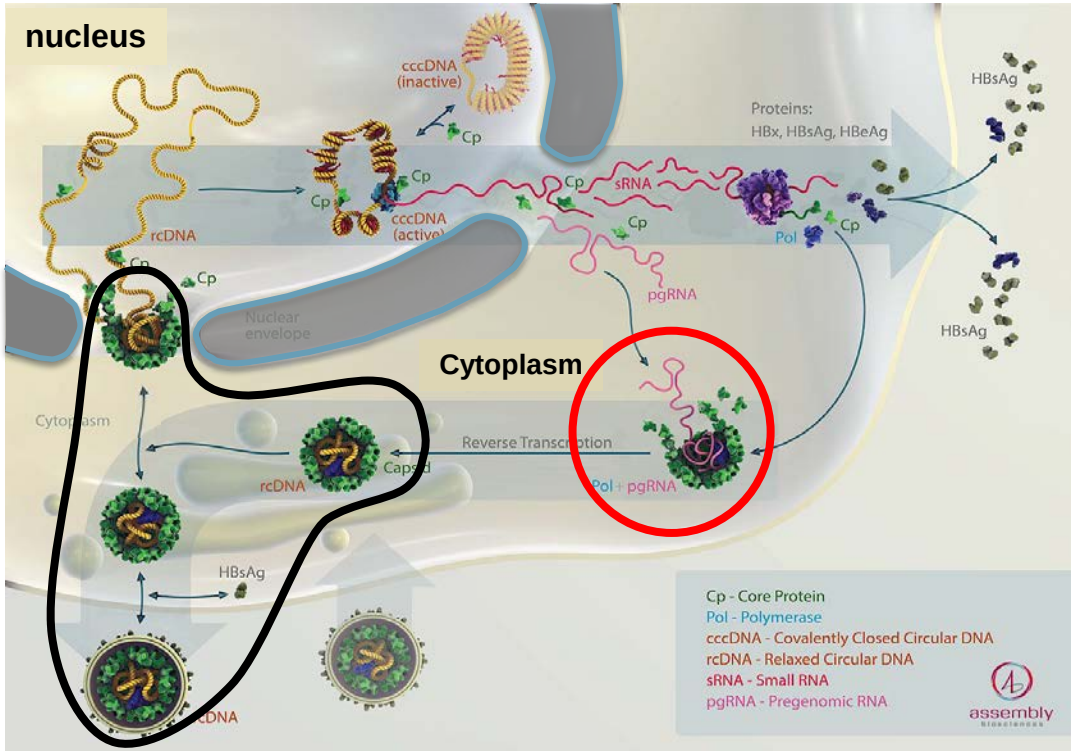




CpAM Dural Mechanisms Of Action: Inhibit Viral Replication and Block cccDNA Formation



CpAMs Target Multiple Steps of HBV Lifecycle



- Core protein plays multiple roles throughout HBV lifecycle and represents an excellent drug target impacting cccDNA levels
- CpAMs represent a new class of direct acting antivirals that are selective for HBV and inhibit *de novo* cccDNA formation
- Assembly Biosciences has established assays to specifically measure cccDNA activities and its first candidate is currently in clinical development

Acknowledgements

Assembly Biosciences HBV Team

Biology

Qi Huang
Dawei Cai
Pao-Chen Li
Emily Connelly
G. Renuka Kumar
Yuhua Zong
Alex Mercier
Xiulan Zhou
Katherine Nabel
Yi Zhou
Ran Yan
Lida Guo
Geoffrey Chen
Esteban Carabajal Ariel
Tang
Uri Lopatin
Richard Colonno

Chemistry

Leping Li
Simon Haydar
Bill Turner
Lynn Bannen
Mark Bures
Samson Francis

Biochemistry

Earl May
Lichun Li
Sara Katen
Steve Dunkelbarger
Jason Deer

assembly biosciences

INDIANA UNIVERSITY
BLOOMINGTON

Adam Zlotnick