Discovery of Potent HIV-1 Capsid Assembly Inhibitors

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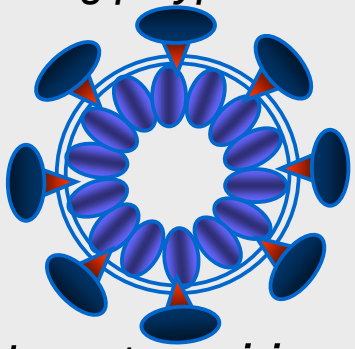
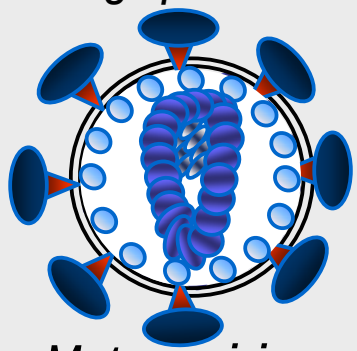
HIV-1 Capsid Assembly and Maturation

Gag polyprotein

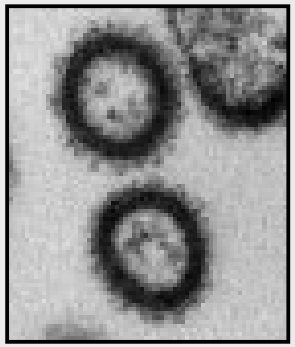
Cleavage products



HIV protease

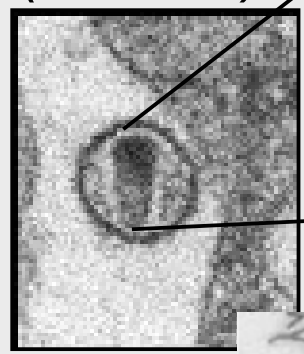


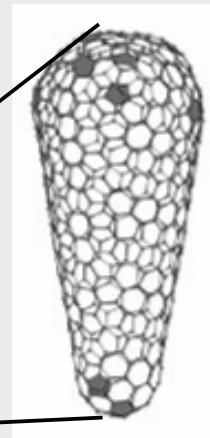
Immature virions (non-infectious)



- Assembly and optimal stability of the core is crucial for viral replication and infectivity

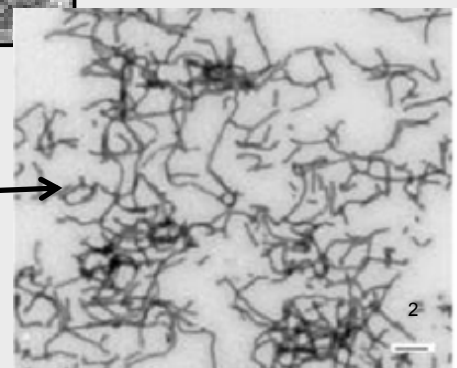
Mature virions (infectious)





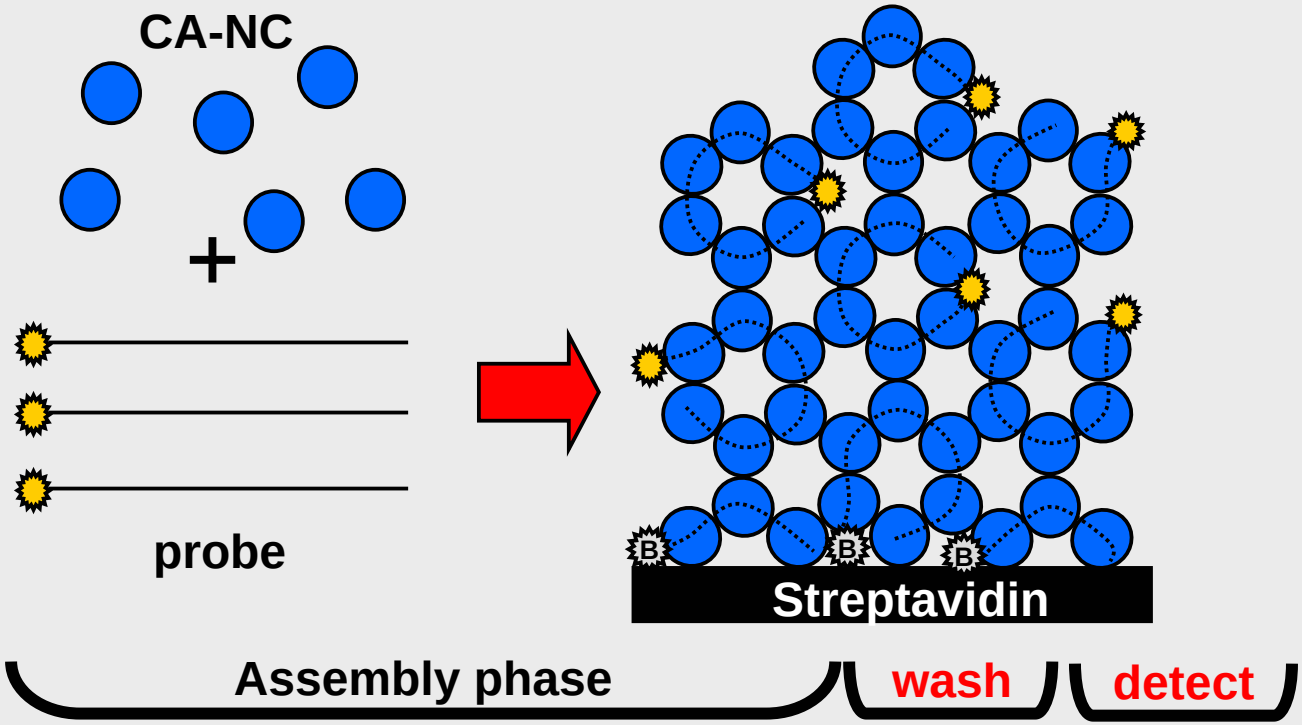
- Purified CA can assemble into core-like particles *in vitro*

EM of capsid tubes assembled *in vitro*



Capsid Assembly Assay (CAA)

In the context of CA-NC, nucleic acid enhances the formation of capsid-like complexes *in vitro*



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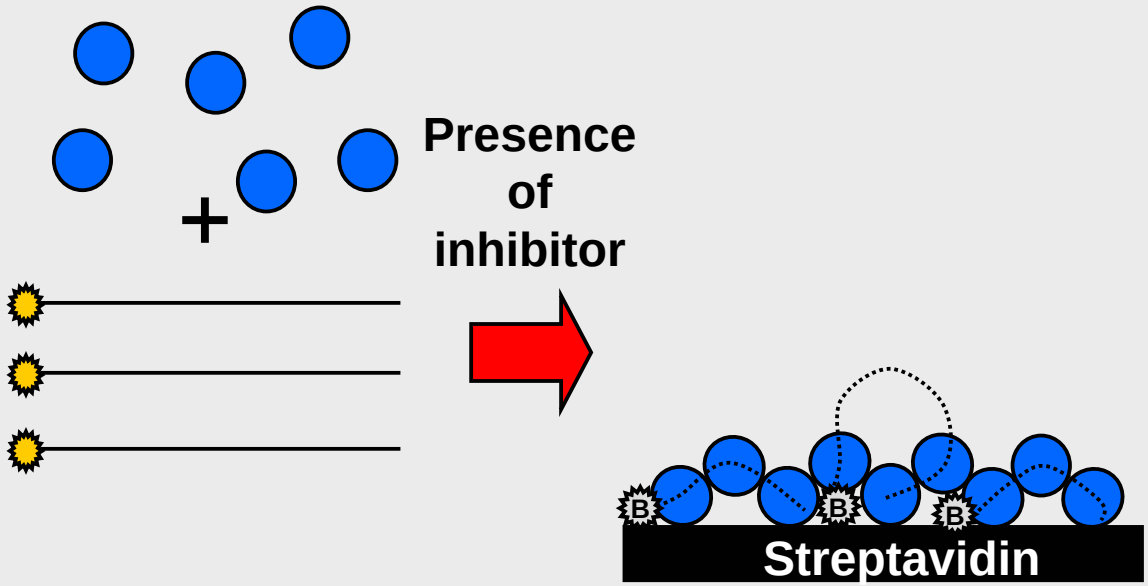


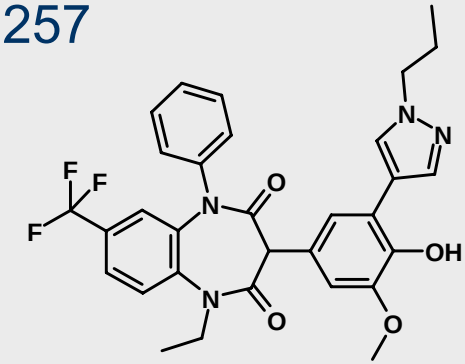
Capsid assembly assay is sensitive to:
- Mutations in both NTD and CTD of CA
- Activity of CAP inhibitors

High Throughput Screening (HTS) and hit analysis

Several chemically distinct clusters of selective hits (chemotypes) were obtained

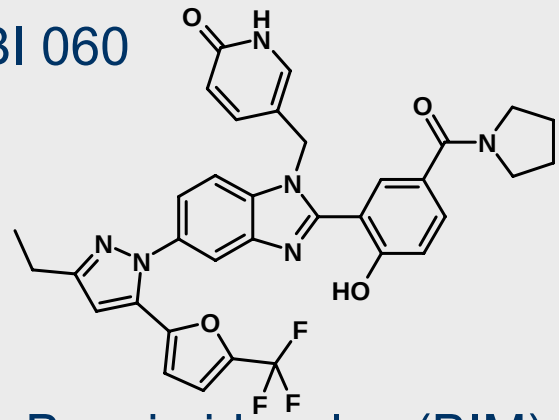
- 2 chemotypes were chosen for Lead Optimization based on multiple parameters including:
  - NMR and co-crystallography → inhibitors bound to CA-NTD

BI 257



Benzodiazepines (BD)

BI 060



Benzimidazoles (BIM)

EC_{50}:	70 ± 30nM (n=21)	62 ± 23 nM (n=53)
CC_{50}:	>28μM	≥20μM

- Resistance selection & Mode-of-Action (MoA) studies were performed
  - MoA was consistent with inhibition of capsid assembly

Cross-resistance profile

Target:		RT	RT	RT	RT	PR	PR	IN
Mutation:	WT	Y188L	V106A	K65R	M184V	V32I /I47V	L33F /I54L	G140S /Q148H
Resistance:		NNRTI	NNRTI	NRTI	NRTI	PI	PI	INSTI
Inhibitor:	EC_{50} (nM)	FC†						
BI 257(BD)	70	1.1	0.9	0.8	0.9	1.5	0.9	1.2
BI 627(BIM)	284	1.0	0.7	1.0	0.6	1.1	1.3	1.2
BI 720(BIM)	112	1.2	0.9	0.7	1.0	0.7	0.6	0.8
nevirapine	18	>83	130*	0.3	0.5	1.4	1.4	1.3
lamivudine	89	1.3	0.9	26.4	>96	0.9	1.2	1.2
amprenavir	35	0.6	0.8	1.2	0.5	6.9	8.3	1.3
raltegravir	1.5	2.8*	0.4	1.0	0.6	0.7	1.8	227

† FC=fold change from matched WT virus

* All values are average of n=2 except: V106A with NVP and Y188L with RAL

- Profile is consistent with a MoA that is distinct from NRTI, NNRTI, PI, INSTI
- Additional MoA studies:
  - inhibitors active in late phase of viral replication cycle

Overview of inhibitor binding within CA pocket

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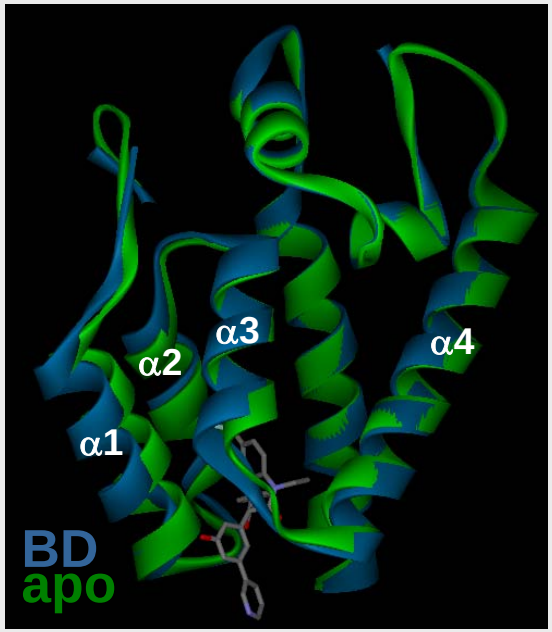
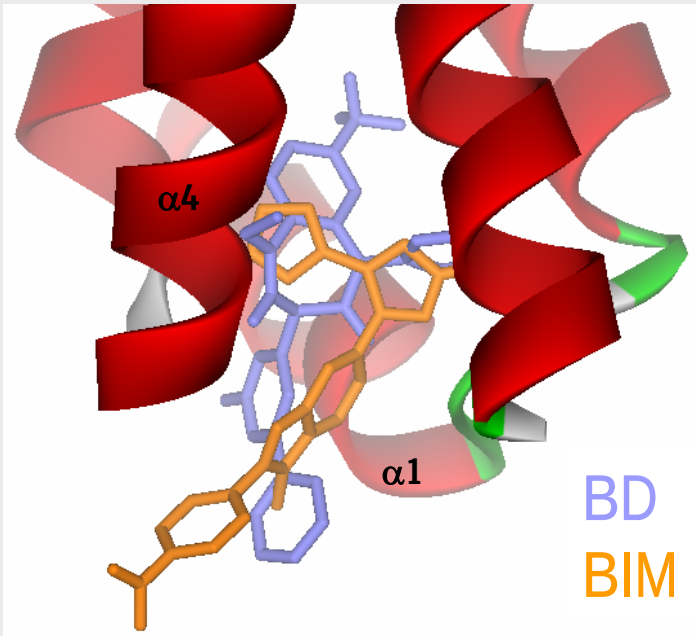
BD chemotype:



- Phe32 moved out of pocket

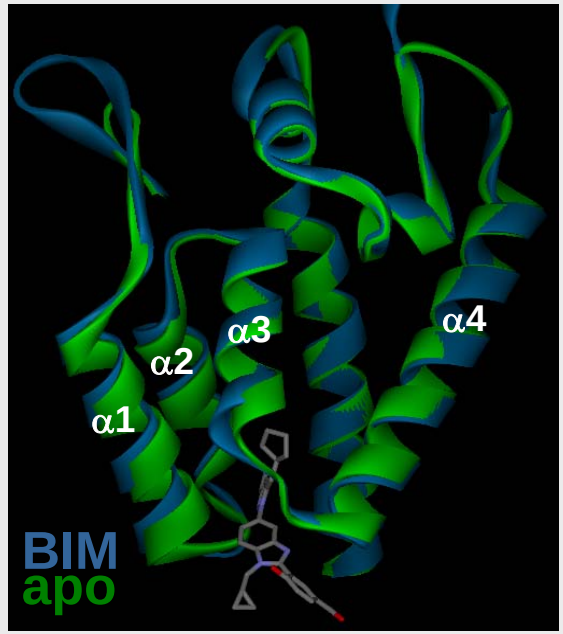
- Helix 1 very shifted
- Inhibitor is bound deep within the helical bundle
- His62 moved out of pocket, backbone NH H-bonds to inhibitor

- Same binding site as CAP inhibitors
- Binding pocket not present in apo-crystal



- Two chemotypes have distinct binding modes and effects of on CA-NTD

BIM chemotype:

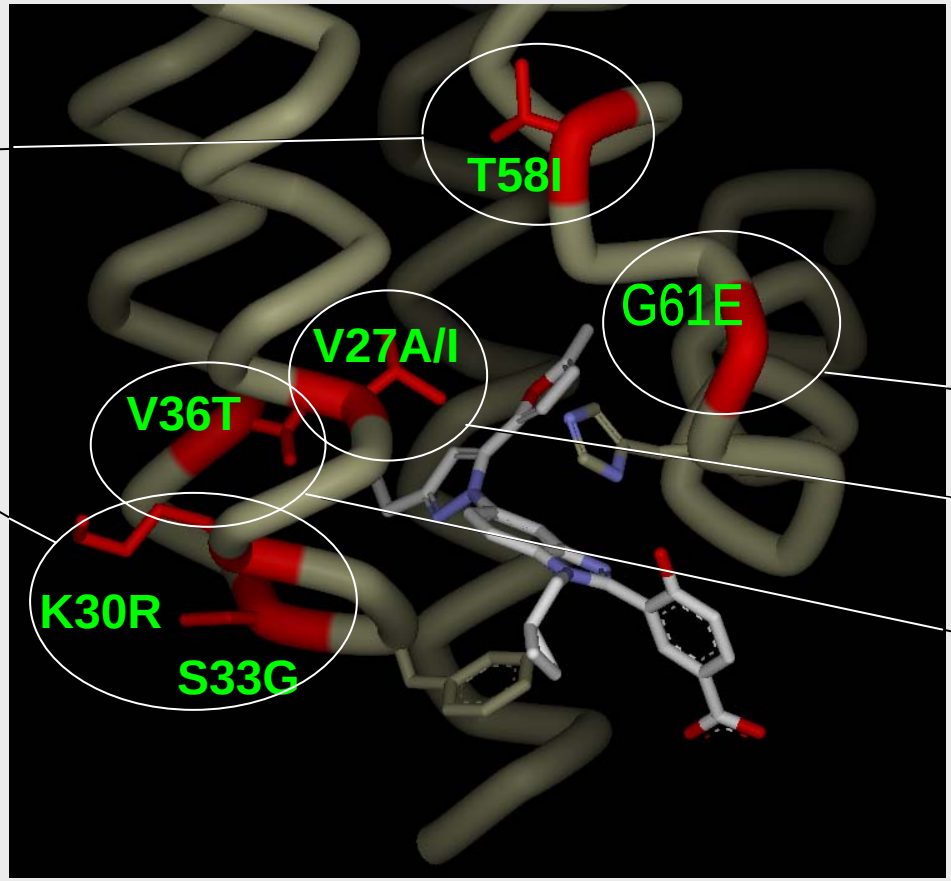


- Phe32 moved out of pocket

- Helices 1 and 4 are less shifted
- Inhibitor is bound less deeply than BD series
- Loop and His62 is in more of an apo-like conformation

Map of resistance mutations

Passage of virus in the presence of CAIs → mutations in CA



- Resistance mutations within the inhibitor binding pocket mapped to helix 1, 2 and 3
- Substitutions in CA CTD (outside of pocket) were selected with high frequency
- Both single and double amino acid substitutions were obtained

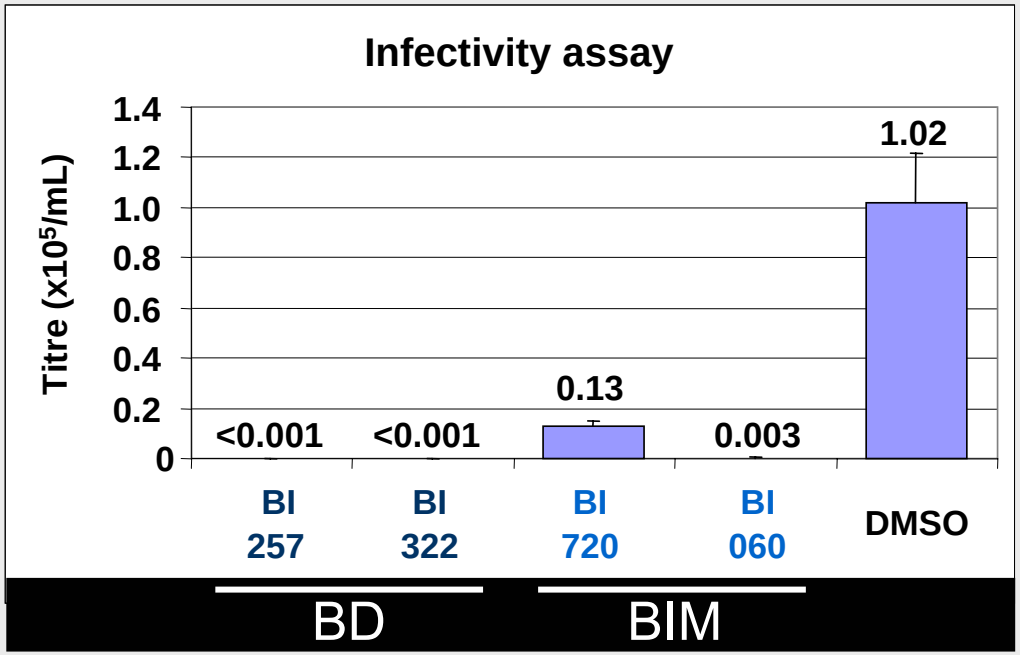
Summary of resistance mutations

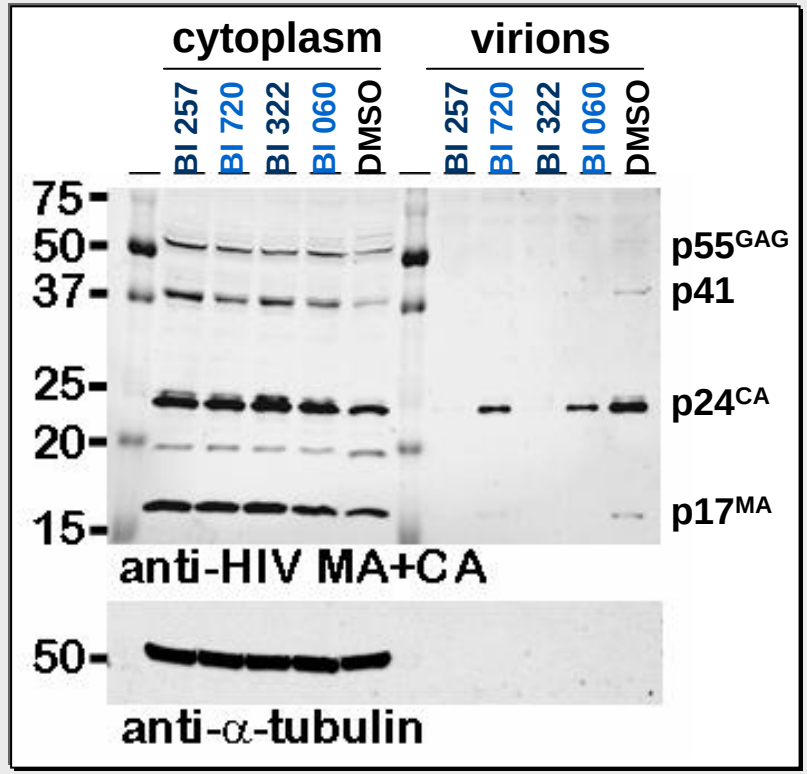
- Cross-resistance against both chemotypes was observed for most mutants
- The majority of mutations selected are rare (non-polymorphic)
- Most capsid assembly inhibitor resistant mutants had reduced replication capacity: From ~3- to >100-fold
- Isothermal titration calorimetry (ITC) studies
  - Some resistance mutations did not affect inhibitor binding (*e.g.* T58I)
  - These same mutations were found to affect the stability of capsid complexes assembled in vitro

→ **Complex resistance profile**

CAIs reduce virus yield or infectivity

Proviral clone used to transfect 293 cells → Inhibitors applied to virus producing cells at 50XEC$_{50}$ → analysis performed 48h post-transfection





- BDs greatly reduce virus production
- Virus produced in the presence of BIM chemotype resulted in reduced infectivity

EM studies reveal MoA of CAIs

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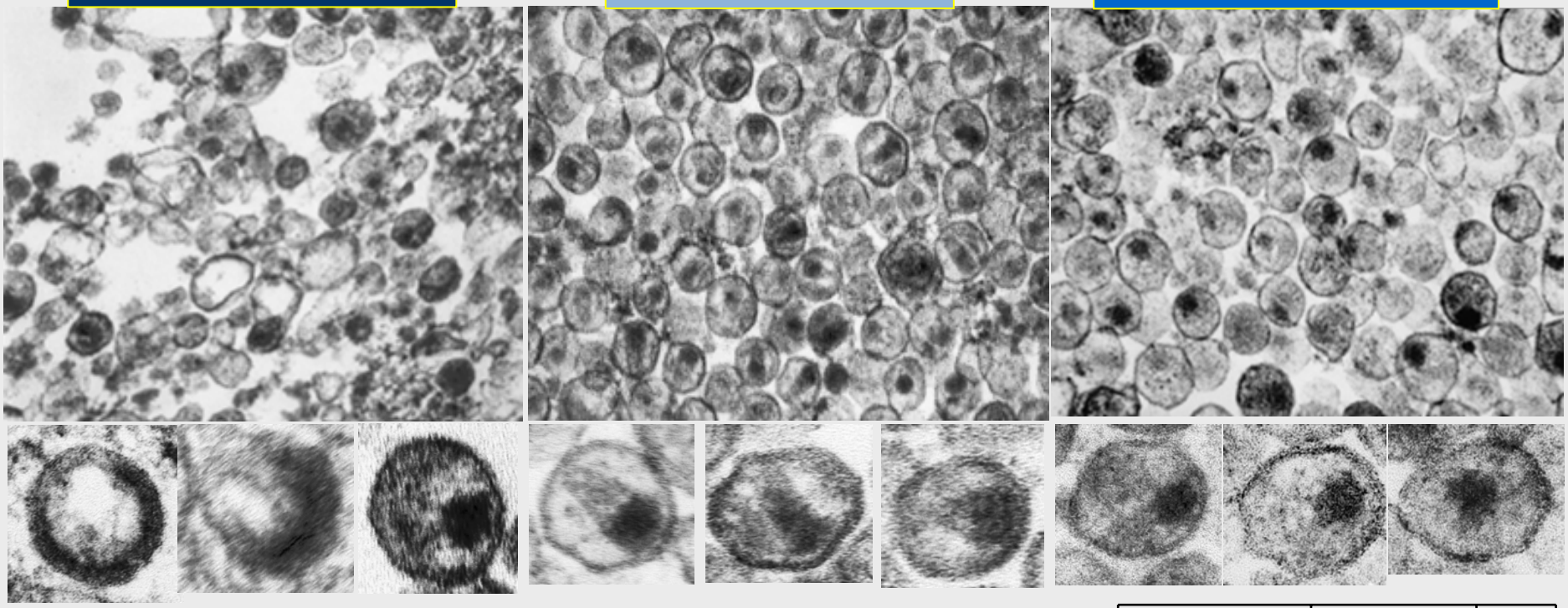
BD | DMSO Control | BIM



- Different chemotypes have distinct morphological effects
  - → BD causes an immature assembly defect
  - → BIM induces a morphological defect in assembly of capsid cores

Treatment	% cones	sd
BIM	2	0.2
BD	2	1.1
DMSO	44	8.7

Structural basis for inhibition of capsid assembly

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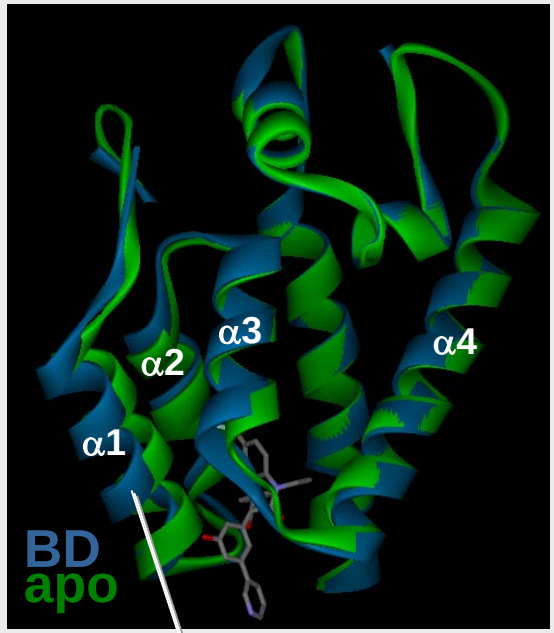
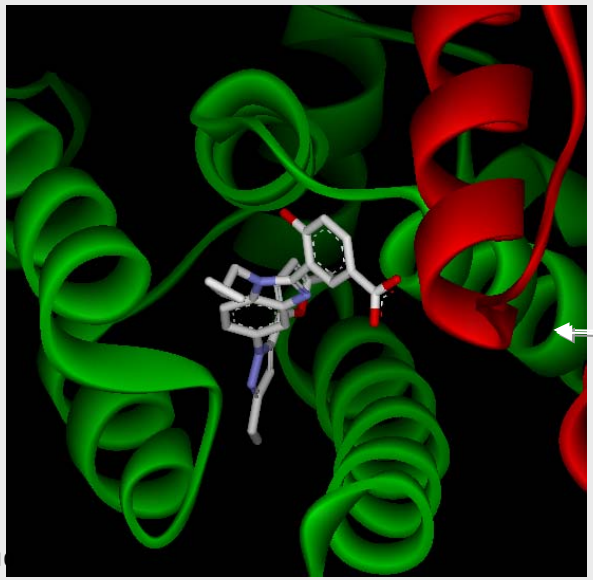
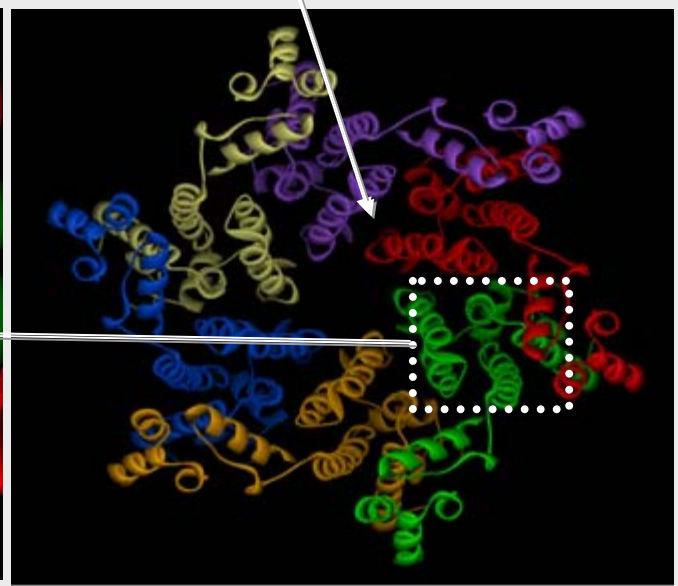
BD chemotype:
- displacement of α1
→ incompatible with packing of NTD within hexamer

BIM chemotype:
- potential for contacts with CTD
→ disrupt interaction between NTD and CTD (intermolecular)







Adapted from Pornillos et al. Cell 137, 1282–1292 (2009)

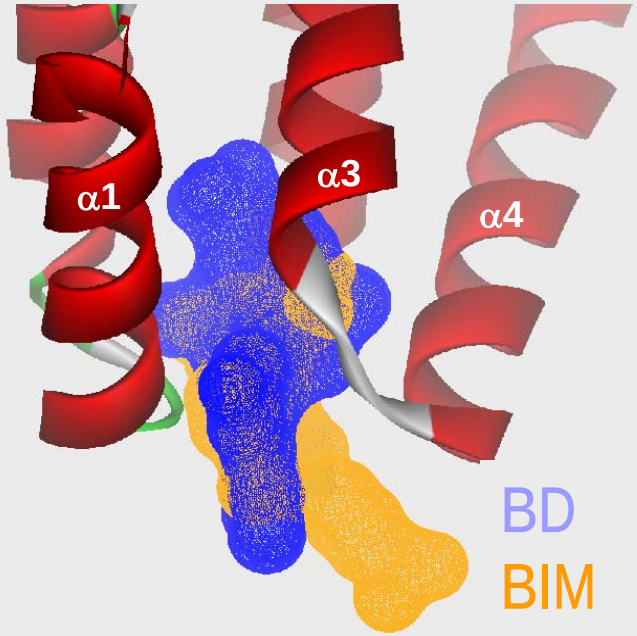
Summary

- Identified two chemotypes that:
  - Inhibit capsid assembly *in vitro*
  - Bind to CA-NTD
  - Inhibit viral replication
- Profile consistent with distinct MoA
  - No cross resistance observed with mutations conferring resistance to NNRTI, NRTI, PI, INI
  - Late antiviral effect
  - Resistance mutations map to CA and affect inhibitor binding or assembly function
  - EM studies demonstrated inhibitors had profound effects in virion production and morphology
- Complex resistance genotype/profile obtained with capsid assembly inhibitors
  - Most mutations in highly conserved residues resulting in reduced replication capacity

Conclusions

We have demonstrated a proof-of-concept for obtaining potent capsid assembly inhibitors toward discovery of new anti-HIV drugs

- Difference in binding between BD and BIM lead to differential effects on selection of resistance mutations and MoA



Significant effort has been invested by BI on inhibitors of capsid assembly

- Several issues could not be reconciled with potency
  - → Highly lipophilic and flexible binding pocket
  - → Lead optimization was terminated

HIV Capsid – *Acknowledgements*

Boehringer Ingelheim (Canada)

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