STRUCTURAL BASIS FOR INHIBITOR-INDUCED AGGREGATION OF HIV INTEGRASE

Abstract #171LB

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Background:
The allosteric integrase (IN) inhibitors (ALLINIs) target the viral-encoded IN protein and interfere with HIV replication via disruption of viral particle assembly late during HIV replication.

Methods:
To investigate their inhibitory mechanism, we crystallized full-length HIV-1 IN bound with the ALLINI GSK1264, and determined the structure of the complete inhibitor interface at 4.4 Å resolution.

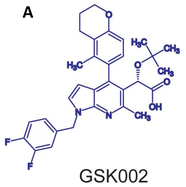
Results:
In this structure, GSK1264 is buried between the catalytic core domain of one IN dimer and the CTD of an adjacent IN dimer. The amino-terminal domain is not resolved in the structure but does not participate in GSK1264 binding. The GSK1264 binding interface is rich in residues implicated in IN oligomerization and ALLINI sensitivity, indicating likely functional significance. The IN-IN interaction mediated by GSK1264 leads to formation of an open polymer in the crystal, a polymerization reaction that is readily reproduced in solution with purified components. To probe ALLINI function more broadly, we compared the properties of several ALLINIs in biochemical, biophysical, virological, and electron microscopic assays. Several ALLINI escape mutations encode IN substitutions at or near the inhibitor binding site, and these also resulted in decreased IN oligomerization in vitro. The results support a mechanism where ALLINIs disrupt viral particle maturation by promoting formation of the IN polymers observed in the IN-GSK1264 crystal structure. Additionally, the results support a structural model for the catalytically inactive IN tetramer discussed in several previous studies. Identification of the interface responsible for polymer formation provides data useful for improving HIV inhibitors and helps explain a wealth of previous studies of HIV IN.

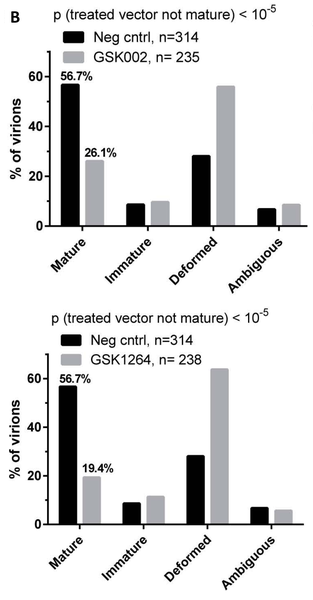
Introduction

- The mechanism of action of strand transfer inhibitors of HIV IN is well known, and these drugs are in widespread clinical use.
- The occurrence of drug resistance necessitates the ongoing development of new classes of compounds that target HIV IN.
- ALLINIs are active against HIV in vitro, and currently being developed for use in patients, motivating further study to guide future inhibitor development.
- IN, which consists of three independently folded domains, catalyzes covalent attachment of viral DNA to host cell DNA; the host factor LEDGF/p75 affects the targeting and efficiency of this reaction.
- Although ALLINIs target IN and were discovered by their ability to block the IN-LEDGF interaction, they interfere only modestly with early steps in the HIV replication cycle.
- The major effect of ALLINIs is observed during particle assembly and maturation. Treatment with ALLINIs results in production of particles with abnormal organization that show reduced infectivity. This effect is accompanied by molecular aggregation of IN.

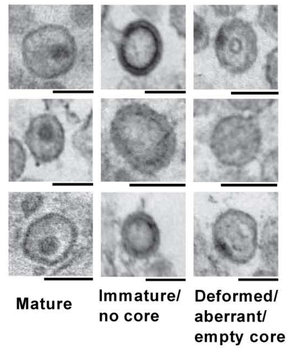
1) The ALLINIs GSK1264 and GSK002 interfere with viral particle assembly

ALLINI treatment of a producer cell line (A1953) significantly reduced the percentage of mature virions and increased the number of deformed virions.



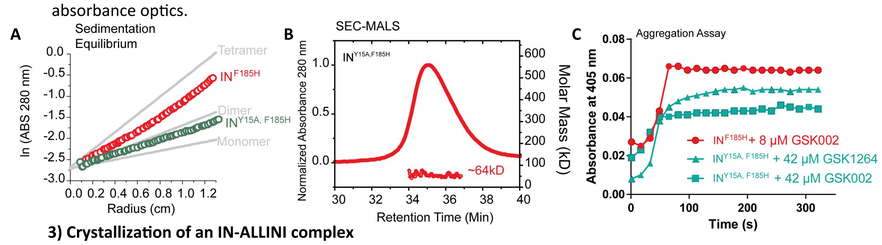


A) Chemical structures of compounds used in this study. **B)** Quantification of morphology of viral particles produced in presence of inhibitors, showing disruption of assembly with ALLINI treatment. **C)** Representative EM micrographs of particle morphologies.



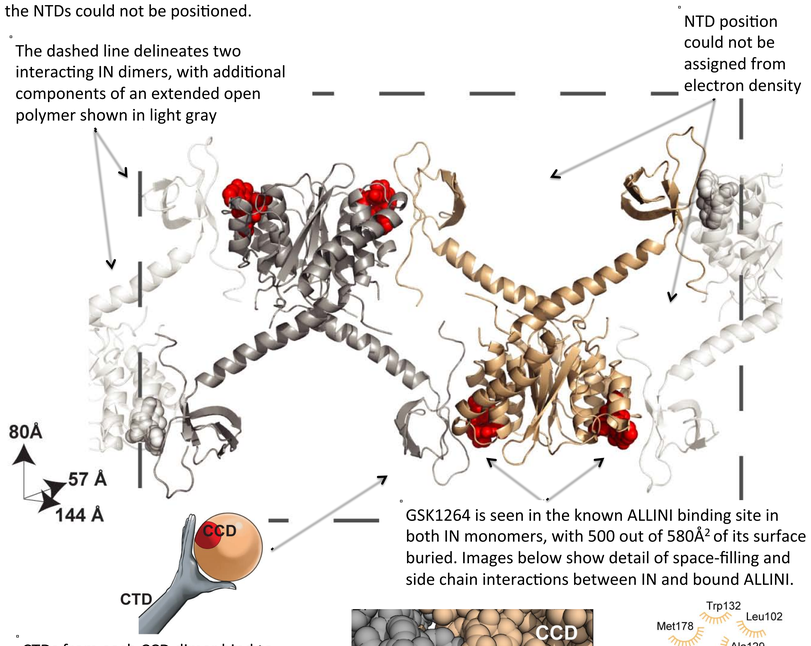
2) Identification of a selectively-aggregating IN construct

We used the ALLINI GSK1264 (which enhances IN aggregation) and solubility-enhancing mutations (which decrease IN aggregation) to control IN self-association. $IN^{Y15A,F185H}$ formed discrete dimers in solution, but retained some ability to aggregate in the presence of GSK1264. **A)** Sedimentation equilibrium analysis of $IN^{Y15A,F185H}$, showing dimeric behavior in solution. **B)** SEC-MALS analysis showing retention time and MW analysis consistent with dimeric IN. **C)** Aggregation of $IN^{Y15A,F185H}$ in the presence of ALLINIs, as measured by absorbance optics.



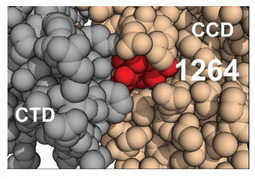
3) Crystallization of an IN-ALLINI complex

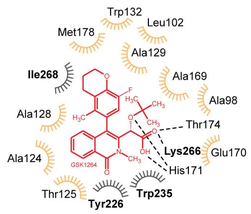
Crystals of $IN^{Y15A,F185H}$ grew in the presence of GSK1264 and diffracted to modest resolution. One IN dimer was observed in the asymmetric unit. The CCDs, CTDs, the CCD-CTD linker, and GSK1264 are well-defined, but the NTDs could not be positioned.



GSK1264 is seen in the known ALLINI binding site in both IN monomers, with 500 out of 580Å^2 of its surface buried. Images below show detail of space-filling and side chain interactions between IN and bound ALLINI.

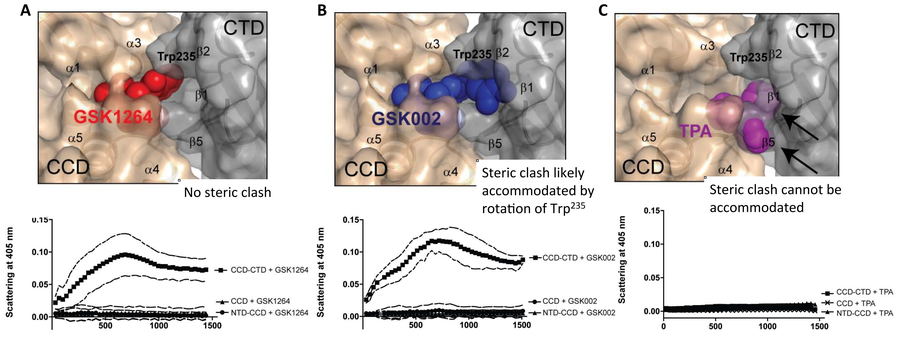
CTDs from each CCD dimer bind to adjacent IN dimers in a manner resembling a hand grasping a ball, with the fingers contacting the ALLINI binding site.





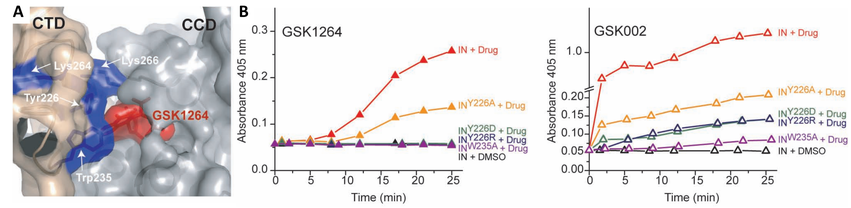
4) Importance of the ALLINI-binding interface

IN constructs containing only the CCD and CTD domains support aggregation by ALLINIs in vitro. IN constructs of the NTD and CCD, or the CCD alone do not support aggregation. Figures **A)**, **B)**, and **C)** show predicted structural arrangement of the CCD-CTD interface with bound GS1264, GSK002, or Tetraphenylarsonium (TPA), respectively. TPA occupies the same site in IN (but is not an ALLINI), and is predicted to clash with the CTD of an adjacent dimer, explaining the lack of aggregation induced by this compound.



5) Substitutions that block ALLINI-Induced Aggregation

Biochemical studies have implicated CTD residues in IN oligomerization, but these substitutions have not been studied in detail. The structure of the IN-GSK1264 complex reveals the role of K264 and K266 in the ALLINI binding interface, and mutation of these residues attenuates aggregation. Two additional CTD residues, Y226 and W235, contribute to the IN-ALLINI interface. As expected, substitutions at these sites inhibit aggregation. **A)** IN CCD-CTD interface with bound GSK1264 showing CTD residues important for ALLINI-induced aggregation. **B)** Aggregation of IN, as measured by absorbance optics, showing decreased ALLINI-induced aggregation of CTD mutants at the CCD-CTD interface.

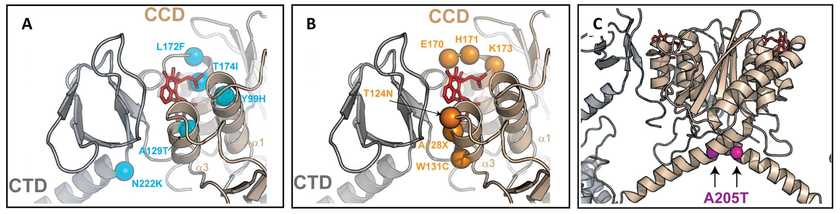


6) Mechanisms of ALLINI resistance

Resistance mutations were identified after serial passage of HIV-1 strains in the presence of increasing concentrations of GSK002 and GSK1264. Mutants showed reduced oligomerization in vitro and caused the following effects:

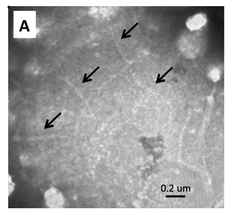
- Direct steric clashes that block inhibitor binding
- Indirect steric clashes due to altered occupied volume of the CCD
- Altered geometry of the alpha-helical CCD-CTD linker
- Altered intersubunit CTD-CCD interface, interfering with oligomerization

A) 1264 Resistance Mutation
B) 002 Resistance Mutation
C) Resistance Mutation for both 002 and 1264



7) Evidence of open polymer formation by EM

ALLINI-induced interdimeric CCD-CTD contacts are expected to form an open polymer of IN dimers, forming large aggregates or fibrils that interfere with particle assembly and maturation. **A)** Solutions containing $IN^{Y15A,F185H}$ and GSK1264 formed fibril-like structures visible by EM.



Key points:

- **The crystal structure of IN-GSK1264 reveals the mechanism of ALLINI-induced aggregation of IN.**
- **GSK1264 is buried in a pocket formed by the CCD of one dimer and the CTD of an adjacent dimer. The pattern of interacting dimers forms an open polymer.**
- **Mutations that disrupt this interface directly or indirectly convey resistance to GSK1264.**
- **We predict that open polymer formation is the cause of ALLINI-induced IN aggregation, and the formation of open polymer fibrils is responsible for disrupting viral particle assembly and maturation in vivo.**

Notes and Acknowledgements
GSK002 and GSK1264 are now owned by ViiV Healthcare, Brentford, UK.
We are grateful for the assistance of the Penn Electron Microscopy Research Laboratory for assistance with acquisition of the EM images.

Crystallographic Data

Data Collection	IN(Y15A,F185H) •GSK1264
PDB	5HOT
Source	ALS 5.0.2
Resolution (Å)	20.0 – 4.4
Space Group	P6$_1$22
Unit-cell	107.06 Å x 107.06 Å x 243.49 Å 90°,90°,120°
Monomers in A.S.U.	2
No. of measured reflections	27,279
No. of unique reflections	5,416

Completeness (%)	93.9 (40.4)
R_{merge} (%)[a]	6.5 (41.2)
Mean I/σ(I)	17 (4.2)
Multiplicity	5.0 (4.8)
R_{free} (%)	36.0
R_{factor} (%)	31.2
R.M.S.D. bond angles (°)	0.007
R.M.S.D. bond lengths (Å)	1.30
Ramachandran Most favored	83.2
Ramachandran Outlier	0.3%