Supplemental material

Novel Small Molecule HIV-1 Replication
Inhibitors that Stabilize Capsid Complexes

Louie Lamorte[1##], Steve Titolo[1], Christopher T. Lemke[2], Nathalie Goudreau[2], Jean-François Mercier[1], Elizabeth Wardrop[1], Vaibhav B. Shah[4], Uta K. von Schwedler[3*], Charles Langelier[3&], Soma S. R. Banik[1δ], Christopher Aiken[4], Wesley I. Sundquist[3], Stephen W. Mason[1@#]

Boehringer Ingelheim (Canada) Ltd., Research & Development, [1]Department of Biological Sciences and [2]Department of Chemistry, Laval, Quebec, Canada; [3]University of Utah, Department of Biochemistry, Salt Lake City, Utah USA; [4]Vanderbilt University School of Medicine, Department of Microbiology and Immunology, Nashville, TN USA

Co-corresponding authors:

RESGeneral.LAV@boehringer-ingelheim.com

Stephen.Mason@bms.com

Present addresses:

& University of California San Francisco, Department of Medicine, CA, USA

δ Integral Molecular, 3711 Market St, Suite 900, Philadelphia, PA, USA

[@] Bristol-Myers Squibb, Wallingford, CT, USA

* Deceased

FIG S1 Stabilization of CA-NC tube-like structures by BI-2. CA assembly assay was performed in solution using 9 µM CA-NC and 1 µM oligonucleotide ($(TG)_{50}$) at different NaCl concentrations (300, 400 and 500 mM) in 100 µl of assay buffer containing 50mM TrisCl pH 8.0 and 5 mM β-mercaptoethanol, in the presence of 5, 20 or 200 µM BI-2 or DMSO, for 16 h at 4°C. CA-NC tubes were pelleted by centrifuging samples for 30 min at 14,000 rpm in a micro-centrifuge. The pellets (P) were solubilized in 5x SDS loading buffer (250 mM TrisCl pH 6.8, 10 % SDS, 0.5% Bromophenol blue, 50 % glycerol and 500 mM β-mercaptoethanol), and the supernatant (S) was adjusted to 1X SDS-PAGE loading buffer (final concentration (50 mM TrisCl pH 6.8, 2 % SDS, 0.1% Bromophenol blue, 10 % glycerol and 100 mM β-mercaptoethanol). Both samples were denatured for 5 min at 95°C and resolved by polyacrylamide gel electrophoresis (12% acrylamide). Gels were fixed and stained with Coomassie Blue. Data shown are representative of 3 independent experiments.

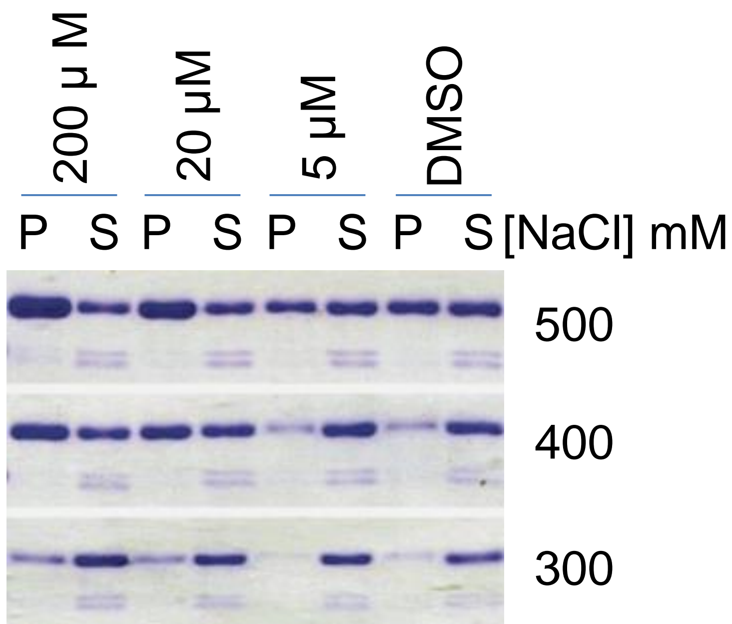

Table S1: Data processing and final model refinement statistics of co-crystal structure of CA_{NTD} in complex with BI-1.

Data Collection	
Space group	$P2_12_12_1$
***a*, *b*, *c* (Å)**	40.78, 42.94, 83.28
α, β, γ (°)	90.0, 90.0, 90.0
Resolution range (Å)	40-1.74 (1.8-1.74)
Completeness (%)	96.6 (93.2)
Redundancy	8.2 (7.1)
$< I / \sigma I >$	21.0
R_{merge}	0.047 (.445)
Refinement	
Resolution range (Å)	29.74-1.73 (1.79-1.73)
No. reflections, working	13491 (1526)
No. reflections, test	1205 (127)
Final R_{cryst}	0.197 (0.413)
Final R_{free}	0.233 (0.422)
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.79
Ramachandran plot	
Favoured regions (%)	98.6
Additionally allowed (%)	1.4

Table S2: Antiviral potency of BI-1 and BI- 2 against NNRTI drug-resistant VSV-G pseudotyped HIV-1 virus.

Virus	wild type	RT K103N/ Y181C	RT V106A	RT Y188L
Inhibitor	EC_{50} (µM)	Average EC_{50} fold change[a]		
BI-1	8.2	0.9	1	1
BI-2	1.8	1	1	1
nevirapine	0.072	>30	>30	>30

[a]The average fold EC_{50} change represents the ratio of the EC_{50} against the mutant virus versus the EC_{50} against the wild type virus. Values represent the average of at least 3 independent experiments.

FIG S2 Bound BI-1 was superposed into the equivalent binding site in hexameric CA (3MGE PDB structure). The surface of hexameric CA, viewed perpendicular to the 6 fold axis, is colored by monomer, with the residues of the N-terminal domain (1 to 146) in lighter shades. Bound BI-1 is shown in white sticks.