Supplementary Materials 2

Reaching beyond HIV/HCV: Nelfinavir as a potential starting point for broad-spectrum protease inhibitors against dengue and chikungunya virus

Soumendranath Bhakat[a*], Leen Delang[b], Suzanne Kaptein[b], Johan Neyts[b*], Pieter Leyssen[b], Venkatesan Jayaprakash[c*]

a Division of Biophysical Chemistry, Lund University, P.O. Box 124, SE-22100 Lund, Sweden

b KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000 Leuven, Belgium

c Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra-835215, India

*Corresponding authors

Soumendranath Bhakat; email: soumendranath.bhakat@bpc.lu.se

Johan Neyts; email: johan.neyts@rega.kuleuven.be

Venkatesan Jayaprakash; email: venkatesanj@bitmesra.ac.in

All MD simulations were performed using GPU version of PMEMD engine integrated with Amber 14. The FF99SB force field was used to describe the protein systems. The partial atomic charges for the ligands were calculated using restraint electrostatic potential procedure at HF/6-31G* level using Gaussian 03 package. The ligands were parametrized using GAFF whereas Leap module was used to add hydrogen's, counter ions to the systems. All the systems were immersed in a 10 angstrom water box filled with TIP3P water molecules. Long-range columbic interactions were treated using particle mesh Ewald (PME) implemented in Amber 14 with a direct space and vdW cut-off of 12 Angstrom. The prepared systems were then subjected to different stages e.g. minimizations, heating and equilibration before proceeding to production. Finally, 30ns molecular dynamics simulations were performed for all the systems (starting with different initial velocities, 30ns*5 for each case) using an NPT ensemble with a target pressure set at 1 bar and constant pressure coupling of 2 ps. The setting for each of these steps are

provide below. MM/GBSA binding free energy analyses were performed for each trajectory using Amber 14 to revalidate the free energy profile.

Table 1. Revalidation of MM/GBSA binding free energy profile for NFV and LPV complexed with DENV NS2B-NS3 protease. 5 different simulations were performed each of 30ns starting with different initial velocity. Finally the binding free energy from 5 different runs were averaged in each case.

Ligand	E_{vdw}	E_{elect}	G_{gas}	G_{solv}	ΔG_{bind}
T1	-46.7256±0.2031 -28.9042±0.5213	-13.0021±0.4012 -8.8953±0.2345	-29.0958±0.3210 -30.2007±0.8011	27.4941±0.2497 9.498±0.3043	-32.2336±0.1255 -28.3015±0.4271
T2	-46.5270±0.1025 -27.9058±0.2916	-12.8912±0.3051 -8.8879±0.2531	-30.0021±0.5210 -30.7287±0.8107	26.4061±0.2394 8.3812±0.2953	-33.0121±0.3025 -28.4125±0.3079
T3	-45.8025±0.2502 -27.9102±0.2851	-12.5215±0.2221 -8.7258±0.3012	-30.2198±0.6128 -32.7031±0.7197	26.0315±0.3052 8.2639±0.2960	-32.2925±0.2521 -28.3721±0.2827
T4	-45.9025±0.2109 -27.9218±0.2716	-13.0021±0.0925 -8.5281±0.2919	-30.2581±0.7028 -31.6215±0.7825	26.1754±0.2804 8.3778±0.3062	-32.7292±0.5921 -28.0721±0.2501
T5	-45.8926±0.3028 -28.7635±0.2074	-12.9218±0.0296 -7.8693±0.2944	-29.9827±0.6271 -30.6493±0.6482	25.9617±0.2057 8.7791±0.2081	-32.8527±0.9028 -27.8537±0.2963
TAvg	-46.1700 -28.2811	-12.8677 -8.5813	-29.9117 -31.1789	26.4138 8.66	-32.6240 -28.2024

¶NFV complexed with CHIKV NSP2 whereas * LPV complexed with CHIKV NSP2.

Table 2. Revalidation of MM/GBSA binding free energy profile for NFV and LPV complexed with CHIKV NSP2 protease. 5 different simulations were performed each of 30ns starting with different initial velocity. Finally the binding free energy from 5 different runs were averaged in each case.

Ligand	E_{vdw}	E_{elect}	G_{gas}	G_{solv}	ΔG_{bind}
T1	-28.8572±0.2720¶ -33.3102±0.2052*	-8.2589±0.2053¶ -4.4092±0.2104*	-31.0018±0.3042¶ -32.1830±0.3172*	12.3336±0.3918¶ 15.2266±0.2302*	-24.7825±0.1051¶ -22.4928±0.1037*
T2	-28.2850±0.2035¶ -33.3225±0.2106*	-8.0018±0.2190¶ -4.4250±0.2110*	-30.9108±0.4210¶ -32.1524±0.3210*	11.4843±0.3780¶ 15.4437±0.2402*	-24.8025±0.2091¶ -22.3038±0.1938*
T3	-31.1928±0.2501¶ -33.9628±0.2282*	-7.3192±0.2309¶ -4.4302±0.2107*	-30.8975±0.4502¶ -32.8482±0.3032*	13.4925±0.3050¶ 15.9538±0.3135	-25.0195±0.2305¶ -22.4392±0.2403*
T4	-31.0152±0.2103¶ -32.9837±0.2903*	-7.4209±0.2032¶ -4.4087±0.2143*	-31.1508±0.4001¶ -31.3395±0.3927*	13.3153±0.3105¶ 15.0097 ±0.3236*	-25.1208±0.2780¶ -22.3827±0.2520*
T5	-31.0232±0.2351¶ -33.3193±0.2496*	-7.5108±0.2142¶ -4.3046±0.2842*	-31.1827±0.4129¶ -31.3842±0.4238*	13.3232±0.3312¶ 15.418±0.3020*	-25.2130±0.2715¶ -22.4821±0.2303*

TAvg	-30.0747¶ -33.3797*	-7.7023¶ -4.3955*	-31.0287¶ -31.9814*	12.7897¶ 15.4103*	-24.9876¶ -22.4201*

¶NFV complexed with CHIKV NSP2 whereas * LPV complexed with CHIKV NSP2.

Table 3. System information for DENV and CHIKV protease complexed with the ligands

Systems	No. of Water Molecules	No. of Counter ions
DENV NS2B-NS3+ligands	26703	8
CHIKV NSP2+ligands	32412	15

Simulation inputs

Partial_Mini.in
Initial minimization of MMP3 (MMMM): solvent molecules and added ions
&cntrl
imin = 1,
maxcyc = 2500,
ncyc = 750,
ntb = 1,
ntr = 1,
cut = 8.0,
/
Hold the Protein fixed
10.0
RES 1 331
END
END

Full_Mini.in
Full minimization of MMP3 (MMMM): protein, solvent molecules and added ions
&cntrl
imin = 1,
maxcyc = 200,
ncyc = 50,
ntb = 1,
ntr = 0,
cut =
8.0,

Drms =
0.0001,

/

END

Heating.in
Heating Step of MMP3 (MMMM): stage-
5
 &cntrl

 imin=0,
 irest=0,
 NTX=1,
 ntb= 1,
 NTPR=500,
 NTWX=500,
 NTWR=500,
 ntr=1,
 Tempi=0.0,
 Temp0=300.0,
 NTT=3,
 gamma_ln=1.0,
 NTC=2,
 NTF=2,
 cut= 8.0,
 nstlim=200000,
 dt=0.002,
/
Keep Protein and inhibitor fixed with weak restraints
10.0
RES 1 331
END
END

equil.in
Equilibration Step of MMP3 (MMMM): stage-1
&cntrl
imin= 0,
irest=1,
NTX=5,
ntb=2,
ntp=1,
PRES0=1.0,
TAUP=2.0,
NTPR=500,

NTWX=500,
ntr=0,
Tempi=300.0,
Temp0=300.0,
NTT=3,
gamma_ln=1.0,
NTC=2,
NTF=2,
cut=8.0,
nstlim=1000000,
dt=0.002
/

md.in

Production Step of MMP3 (MMMM): stage-1
&cntrl
imin= 0,
irest=1, ig=-1
ioutfm=1,
ntxo=2,
NTX=5,
ntb=2,
ntp=1,
PRES0=1.0,
TAUP=2.0,
NTPR=500,
NTWX=5000,
ntr=0,
Tempi=300.0,
Temp0=300.0,
NTT=3,
gamma_ln=1.0,
NTC=2,
NTF=2,
cut=12.0,
nstlim=15000000,
dt=0.002
/