In Silico Validation of Allosteric Inhibitors Targeting Zika Virus NS2B-NS3 Proteases

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Table S1. Comparison of GaMD simulation time periods for modeling protein binding pocket conformational changes with and without inhibitors, based on the GaMD calculation method described by Miao Y et al. (DOI: 10.1002/wcms.1521). The table shows the recommended simulation time periods for proteins of various sizes, including the Zika NS2B-NS3 protease (~210 amino acid residues), which requires a 1000 ns production GaMD simulation time period for reliable modeling of conformational changes.

TITLE	Protein/amino residues number	Production GaMD simulation time period	DOI
Remarkable similarity in Plasmodium falciparum and Plasmodium vivax geranylgeranyl diphosphate synthase dynamics and its implication for antimalarial drug design	Homology modelling based on the two proteins (PDB ID: 3MAV and 3PH7)/~378	100 ns	doi.org/10.1111/cbdd.13170
Active Site Breathing of Human Alkbh5 Revealed by Solution NMR and Accelerated Molecular Dynamics	apo Alkbh5 (PDB ID: 4NJ4)/~222	200 ns	10.1016/j.bpj.2018.10.004

Energy landscape of the domain movement in Staphylococcus aureus UDP-N-acetylglucosamine 2-epimerase	S. aureus MnaA-UDP co-structure (PDB ID: 5ENZ) / ~376	250 ns	10.1016/j.jsb.2019.05.004
Schisandrin C targets Keap1 and attenuates oxidative stress by activating Nrf2 pathway in Ang II-challenged vascular endothelium	murine Keap1 in complex with RA839(PDB ID: 5CGJ) / ~289	500 ns	10.1002/ptr.6271
Structural Consequences of Multisite Phosphorylation in the BAK1 Kinase Domain	BAK1 kinase domain (PDB ID: 3TL8, chain A)/ ~300	500 ns	10.1016/j.bpj.2019.12.026
Casein kinase 1 dynamics underlie substrate selectivity and the PER2 circadian phosphoswitch	CASEIN KINASE (PDB ID: 1CKJ, 1CKJ, 6PXN, 6PXN, 1CKJ and 6PXN)/ ~600	500 ns	10.7554/eLife.52343
Elucidation of Cryptic and Allosteric Pockets within the SARS-CoV-2 Main Protease	SARS-CoV-2 Main Protease (PDB ID: 6LU7)/~306	1000 and 200 ns	10.1021/acs.jcim.1c00140
Probing the Molecular Mechanism of	M.tuberculosis RNAP with rifampin (PDB	900 ns	doi/10.1128/aac.02476-19

Rifampin Resistance Caused by the Point Mutations S456L and D441V on Mycobacterium tuberculosis RNA Polymerase through Gaussian Accelerated Molecular Dynamics Simulation	ID 5UHC)/ ~3000		
Dynamics and Location of the Allosteric Midazolam Site in Cytochrome P4503A4 in Lipid Nanodiscs	Human Microsomal P450 3A4 (PDB ID: 1TQN)/ ~371	100 ns	10.1021/acs.biochem.9b01001
This manuscript	Zika NS2B-NS3 protease (PDB ID: 5T1V)/ ~210	1000 ns	This manuscript

- Reference to Miao Y et al. (DOI: 10.1002/wcms.1521)

Table S2. Coordinates of the atoms within the 5 Å cluster system surrounding the inhibitor-01 (StateII) of the Zika NS2B-NS3 protease. The coordinates are provided in Angstroms (Å) and represent the atoms' positions in the protein's three-dimensional structure.

ATOM	1	N	UNL	1	19.877	51.789	62.955	1.00	0.00	N
ATOM	2	C	UNL	1	18.392	50.889	66.651	1.00	0.00	C
ATOM	3	O	UNL	1	20.599	51.197	60.194	1.00	0.00	O
ATOM	4	C1	UNL	1	19.318	51.931	66.595	1.00	0.00	C
ATOM	5	O1	UNL	1	18.937	49.715	60.022	1.00	0.00	O
ATOM	6	C2	UNL	1	19.830	52.306	65.358	1.00	0.00	C
ATOM	7	O2	UNL	1	18.670	47.761	62.131	1.00	0.00	O
ATOM	8	C3	UNL	1	19.410	51.633	64.203	1.00	0.00	C
ATOM	9	O3	UNL	1	12.525	47.524	63.920	1.00	0.00	O
ATOM	10	C4	UNL	1	18.625	50.537	64.273	1.00	0.00	C
ATOM	11	O4	UNL	1	13.263	49.929	62.857	1.00	0.00	O
ATOM	12	C5	UNL	1	18.078	50.119	65.511	1.00	0.00	C
ATOM	13	O5	UNL	1	14.463	45.701	64.863	1.00	0.00	O
ATOM	14	C6	UNL	1	19.305	50.766	62.165	1.00	0.00	C
ATOM	15	C7	UNL	1	18.486	49.962	62.973	1.00	0.00	C
ATOM	16	C8	UNL	1	19.742	50.528	60.786	1.00	0.00	C
ATOM	17	C9	UNL	1	19.211	49.565	58.623	1.00	0.00	C
ATOM	18	C10	UNL	1	17.953	48.585	62.667	1.00	0.00	C
ATOM	19	C11	UNL	1	16.587	48.366	63.239	1.00	0.00	C
ATOM	20	C12	UNL	1	16.256	47.119	63.881	1.00	0.00	C
ATOM	21	C13	UNL	1	14.874	46.867	64.214	1.00	0.00	C
ATOM	22	C14	UNL	1	13.896	47.797	63.855	1.00	0.00	C
ATOM	23	C15	UNL	1	14.228	49.062	63.285	1.00	0.00	C
ATOM	24	C16	UNL	1	15.612	49.354	63.122	1.00	0.00	C
ATOM	25	C17	UNL	1	15.220	45.311	65.980	1.00	0.00	C
ATOM	26	C18	UNL	1	12.462	49.561	61.763	1.00	0.00	C
ATOM	27	Cl	UNL	1	17.834	50.318	68.222	1.00	0.00	CL
ATOM	28	H	UNL	1	19.506	52.532	67.480	1.00	0.00	H
ATOM	29	H1	UNL	1	20.575	53.086	65.225	1.00	0.00	H
ATOM	30	H2	UNL	1	17.696	49.114	65.666	1.00	0.00	H
ATOM	31	H3	UNL	1	20.553	52.466	62.633	1.00	0.00	H
ATOM	32	H4	UNL	1	18.556	48.764	58.259	1.00	0.00	H
ATOM	33	H5	UNL	1	20.225	49.160	58.518	1.00	0.00	H

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ATOM     34  H6  UNL     1      19.217  50.516  58.077  1.00  0.00           H
ATOM     35  H7  UNL     1      17.004  46.335  63.946  1.00  0.00           H
ATOM     36  H8  UNL     1      15.924  50.340  62.790  1.00  0.00           H
ATOM     37  H9  UNL     1      12.480  46.552  63.949  1.00  0.00           H
ATOM     38 H10  UNL     1      15.094  46.137  66.691  1.00  0.00           H
ATOM     39 H11  UNL     1      16.288  45.219  65.748  1.00  0.00           H
ATOM     40 H12  UNL     1      14.840  44.345  66.335  1.00  0.00           H
ATOM     41 H13  UNL     1      11.888  48.667  62.033  1.00  0.00           H
ATOM     42 H14  UNL     1      13.104  49.337  60.903  1.00  0.00           H
ATOM     43 H15  UNL     1      11.748  50.353  61.506  1.00  0.00           H
ATOM     44  CA  LYS   138      17.323  56.994  67.047  1.00  0.00           C
ATOM     45  C   LYS   138      18.060  56.065  66.060  1.00  0.00           C
ATOM     46  O   LYS   138      13.378  52.809  66.779  1.00  0.00           H
ATOM     57  N   GLN   139      13.219  54.231  61.318  1.00  0.00           H
ATOM     72  N   ASP   140      20.029  56.816  63.275  1.00  0.00           N
ATOM     73  CB  ASP   140      22.479  56.740  62.937  1.00  0.00           C
ATOM     74  CG  ASP   140      22.690  56.642  64.474  1.00  0.00           C
ATOM     75 OD1  ASP   140      22.858  55.549  64.981  1.00  0.00           O
ATOM     76  H   ASP   140      19.314  57.437  63.626  1.00  0.00           H
ATOM     77 HB2  ASP   140      22.542  55.737  62.516  1.00  0.00           H
ATOM     78  CG  LEU   141      18.574  55.115  59.509  1.00  0.00           C
ATOM     79 CD1  LEU   141      17.428  54.221  59.278  1.00  0.00           C
ATOM     80 CD2  LEU   141      19.839  54.538  58.948  1.00  0.00           C
ATOM     81  HG  LEU   141      18.712  55.156  60.589  1.00  0.00           H
ATOM     82 HD11 LEU   141      17.292  54.128  58.200  1.00  0.00           H
ATOM     83 HD12 LEU   141      17.652  53.268  59.756  1.00  0.00           H
ATOM     84 HD21 LEU   141      19.660  54.550  57.873  1.00  0.00           H
ATOM     85 HD22 LEU   141      20.740  55.096  59.204  1.00  0.00           H
ATOM     86 HD23 LEU   141      20.011  53.505  59.248  1.00  0.00           H
ATOM     87 CE2  TRP   148      16.017  52.309  55.339  1.00  0.00           C
ATOM     88 NE1  TRP   148      15.440  52.627  56.496  1.00  0.00           N
ATOM     89 CZ2  TRP   148      16.870  51.306  54.939  1.00  0.00           C
ATOM     90 CH2  TRP   148      17.301  51.292  53.577  1.00  0.00           C
ATOM     91 HE1  TRP   148      15.541  52.129  57.369  1.00  0.00           H
ATOM     92 HZ2  TRP   148      17.268  50.646  55.697  1.00  0.00           H
ATOM     93 HH2  TRP   148      18.037  50.581  53.234  1.00  0.00           H
ATOM     94  CG  LEU   150      13.009  49.104  56.408  1.00  0.00           C
ATOM     95 CD1  LEU   150      13.200  49.034  57.914  1.00  0.00           C
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ATOM     96  CD2 LEU   150      14.375  48.876  55.791  1.00  0.00           C
ATOM     97  HB2 LEU   150      10.945  48.604  56.623  1.00  0.00           H
ATOM     98  HG  LEU   150      12.767  50.161  56.304  1.00  0.00           H
ATOM     99 HD11 LEU   150      13.825  49.869  58.231  1.00  0.00           H
ATOM    100 HD12 LEU   150      12.227  49.264  58.349  1.00  0.00           H
ATOM    101 HD13 LEU   150      13.579  48.068  58.247  1.00  0.00           H
ATOM    102 HD21 LEU   150      14.790  47.885  55.974  1.00  0.00           H
ATOM    103 HD23 LEU   150      15.140  49.612  56.034  1.00  0.00           H
ATOM    104  CB  THR   183      20.338  47.514  71.015  1.00  0.00           C
ATOM    105  CG2 THR   183      19.011  47.563  70.273  1.00  0.00           C
ATOM    106  OG1 THR   183      20.788  48.811  71.044  1.00  0.00           O
ATOM    107  HG1 THR   183      20.911  49.064  70.126  1.00  0.00           H
ATOM    108 HG21 THR   183      18.127  47.931  70.793  1.00  0.00           H
ATOM    109 HG22 THR   183      19.151  48.050  69.308  1.00  0.00           H
ATOM    110 HG23 THR   183      18.665  46.559  70.031  1.00  0.00           H
ATOM    111  CA  ASP   185      15.851  49.840  72.675  1.00  0.00           C
ATOM    112  CB  ASP   185      15.830  50.688  71.360  1.00  0.00           C
ATOM    113  CG  ASP   185      16.648  51.927  71.458  1.00  0.00           C
ATOM    114  OD1 ASP   185      17.827  51.927  71.128  1.00  0.00           O
ATOM    115  OD2 ASP   185      16.134  52.988  71.961  1.00  0.00           O
ATOM    116  H   ASP   185      17.973  50.062  72.578  1.00  0.00           H
ATOM    117  HB2 ASP   185      16.220  50.053  70.565  1.00  0.00           H
ATOM    118  HB3 ASP   185      14.793  50.964  71.175  1.00  0.00           H
ATOM    119  HA3 GLY   186      14.517  45.969  71.605  1.00  0.00           H
ATOM    120  N   ASP   187      16.422  44.769  70.409  1.00  0.00           N
ATOM    121  CA  ASP   187      17.211  43.819  69.626  1.00  0.00           C
ATOM    122  C   ASP   187      17.880  44.449  68.420  1.00  0.00           C
ATOM    123  O   ASP   187      17.350  45.474  67.902  1.00  0.00           O
ATOM    124  CB  ASP   187      16.472  42.496  69.238  1.00  0.00           C
ATOM    125  CG  ASP   187      15.719  41.739  70.383  1.00  0.00           C
ATOM    126  OD2 ASP   187      14.501  41.584  70.261  1.00  0.00           O
ATOM    127  H   ASP   187      15.635  45.139  69.895  1.00  0.00           H
ATOM    128  HB2 ASP   187      15.709  42.687  68.483  1.00  0.00           H
ATOM    129  HB3 ASP   187      17.112  41.762  68.748  1.00  0.00           H
ATOM    130  N   ILE   188      18.905  43.808  67.902  1.00  0.00           N
ATOM    131  CA  ILE   188      19.752  44.331  66.833  1.00  0.00           C
ATOM    132  C   ILE   188      20.684  43.290  66.188  1.00  0.00           C
ATOM    133  CB  ILE   188      20.618  45.584  67.258  1.00  0.00           C
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ATOM    134  CG1 ILE   188      21.277  46.479  66.154  1.00  0.00           C
ATOM    135  CD1 ILE   188      20.160  47.205  65.302  1.00  0.00           C
ATOM    136  H   ILE   188      19.220  42.913  68.247  1.00  0.00           H
ATOM    137  HA  ILE   188      19.075  44.691  66.059  1.00  0.00           H
ATOM    138  HB  ILE   188      20.020  46.281  67.845  1.00  0.00           H
ATOM    139 HG12 ILE   188      21.884  47.249  66.630  1.00  0.00           H
ATOM    140 HD11 ILE   188      20.304  48.283  65.374  1.00  0.00           H
ATOM    141 HD12 ILE   188      20.272  46.807  64.293  1.00  0.00           H
ATOM    142 HD13 ILE   188      19.227  47.038  65.840  1.00  0.00           H
ATOM    143  H   GLY   210      20.693  44.340  64.532  1.00  0.00           H
ATOM    144 HG13 VAL   211      18.532  47.692  53.757  1.00  0.00           H
ATOM    145  C   ILE   212      18.431  45.372  56.298  1.00  0.00           C
ATOM    146  O   ILE   212      18.200  45.217  57.478  1.00  0.00           O
ATOM    147  HA  ILE   212      16.476  45.375  55.512  1.00  0.00           H
ATOM    148  N   GLY   213      19.550  45.952  55.886  1.00  0.00           N
ATOM    149  CA  GLY   213      20.531  46.490  56.844  1.00  0.00           C
ATOM    150  C   GLY   213      21.637  47.280  56.158  1.00  0.00           C
ATOM    151  O   GLY   213      21.567  47.592  54.930  1.00  0.00           O
ATOM    152  H   GLY   213      19.749  45.899  54.897  1.00  0.00           H
ATOM    153 HA2  GLY   213      20.044  47.052  57.640  1.00  0.00           H
ATOM    154 HA3  GLY   213      20.920  45.603  57.344  1.00  0.00           H
ATOM    155  N   LEU   214      22.633  47.604  56.988  1.00  0.00           N
ATOM    156  CA  LEU   214      23.639  48.637  56.662  1.00  0.00           C
ATOM    157  C   LEU   214      24.899  48.251  57.363  1.00  0.00           C
ATOM    158  O   LEU   214      24.971  48.154  58.595  1.00  0.00           O
ATOM    159  CB  LEU   214      23.129  50.048  57.057  1.00  0.00           C
ATOM    160  CG  LEU   214      21.950  50.615  56.170  1.00  0.00           C
ATOM    161 CD1 LEU   214      21.529  51.943  56.724  1.00  0.00           C
ATOM    162 CD2 LEU   214      22.555  50.916  54.818  1.00  0.00           C
ATOM    163  H   LEU   214      22.502  47.390  57.967  1.00  0.00           H
ATOM    164  HA  LEU   214      23.810  48.512  55.593  1.00  0.00           H
ATOM    165 HB2 LEU   214      22.781  50.006  58.089  1.00  0.00           H
ATOM    166 HB3 LEU   214      23.985  50.722  57.080  1.00  0.00           H
ATOM    167  HG  LEU   214      21.089  49.950  56.108  1.00  0.00           H
ATOM    168 HD11 LEU   214      20.888  51.816  57.597  1.00  0.00           H
ATOM    169 HD12 LEU   214      22.460  52.419  57.029  1.00  0.00           H
ATOM    170 HD13 LEU   214      21.116  52.445  55.849  1.00  0.00           H
ATOM    171 HD23 LEU   214      21.763  51.378  54.228  1.00  0.00           H
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ATOM    172  CA  ASN   217      24.725  50.673  61.136  1.00  0.00           C
ATOM    173  C   ASN   217      23.573  49.757  61.633  1.00  0.00           C
ATOM    174  O   ASN   217      22.869  50.214  62.500  1.00  0.00           O
ATOM    175  CB  ASN   217      24.335  51.789  60.211  1.00  0.00           C
ATOM    176  CG  ASN   217      23.802  52.904  61.155  1.00  0.00           C
ATOM    177  ND2 ASN   217      22.529  53.111  61.153  1.00  0.00           N
ATOM    178  OD1 ASN   217      24.577  53.574  61.795  1.00  0.00           O
ATOM    179  HA  ASN   217      25.091  51.136  62.053  1.00  0.00           H
ATOM    180  HB2 ASN   217      25.145  52.251  59.646  1.00  0.00           H
ATOM    181  HB3 ASN   217      23.651  51.541  59.399  1.00  0.00           H
ATOM    182 HD21 ASN   217      22.129  53.758  61.819  1.00  0.00           H
ATOM    183 HD22 ASN   217      21.935  52.499  60.613  1.00  0.00           H
ATOM    184  N   GLY   218      23.523  48.531  61.115  1.00  0.00           N
ATOM    185  CA  GLY   218      22.802  47.408  61.714  1.00  0.00           C
ATOM    186  C   GLY   218      21.864  46.577  60.785  1.00  0.00           C
ATOM    187  O   GLY   218      21.894  46.770  59.567  1.00  0.00           O
ATOM    188  H   GLY   218      24.140  48.306  60.348  1.00  0.00           H
ATOM    189  HA2 GLY   218      23.418  46.690  62.255  1.00  0.00           H
ATOM    190  HA3 GLY   218      22.047  47.749  62.421  1.00  0.00           H
ATOM    191  N   VAL   219      21.185  45.556  61.306  1.00  0.00           N
ATOM    192  CA  VAL   219      20.285  44.648  60.602  1.00  0.00           C
ATOM    193  C   VAL   219      18.893  44.739  61.302  1.00  0.00           C
ATOM    194  O   VAL   219      18.755  44.609  62.499  1.00  0.00           O
ATOM    195  H   VAL   219      21.360  45.439  62.294  1.00  0.00           H
ATOM    196  HA  VAL   219      20.124  44.956  59.569  1.00  0.00           H
ATOM    197  N   VAL   220      17.829  44.904  60.547  1.00  0.00           N
ATOM    198  CA  VAL   220      16.480  45.018  61.063  1.00  0.00           C
ATOM    199  C   VAL   220      15.934  43.688  61.370  1.00  0.00           C
ATOM    200  O   VAL   220      16.155  42.788  60.588  1.00  0.00           O
ATOM    201  CB  VAL   220      15.649  45.781  60.017  1.00  0.00           C
ATOM    202  CG1 VAL   220      14.164  45.897  60.424  1.00  0.00           C
ATOM    203  CG2 VAL   220      16.271  47.140  59.719  1.00  0.00           C
ATOM    204  H   VAL   220      17.891  44.961  59.540  1.00  0.00           H
ATOM    205  HA  VAL   220      16.533  45.598  61.985  1.00  0.00           H
ATOM    206  HB  VAL   220      15.715  45.150  59.131  1.00  0.00           H
ATOM    207 HG11 VAL   220      13.652  44.963  60.650  1.00  0.00           H
ATOM    208 HG12 VAL   220      14.031  46.567  61.274  1.00  0.00           H
ATOM    209 HG13 VAL   220      13.632  46.449  59.649  1.00  0.00           H
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ATOM    210 HG21 VAL   220      15.814  47.370  58.756  1.00  0.00           H
ATOM    211 HG22 VAL   220      15.993  47.891  60.459  1.00  0.00           H
ATOM    212 HG23 VAL   220      17.360  47.175  59.702  1.00  0.00           H
ATOM    213  N   ILE   221      15.383  43.482  62.553  1.00  0.00           N
ATOM    214  CA  ILE   221      15.026  42.158  63.081  1.00  0.00           C
ATOM    215  C   ILE   221      13.577  41.826  62.748  1.00  0.00           C
ATOM    216  O   ILE   221      12.713  42.692  62.971  1.00  0.00           O
ATOM    217  CB  ILE   221      15.361  41.955  64.584  1.00  0.00           C
ATOM    218  CG1 ILE   221      16.814  42.158  64.901  1.00  0.00           C
ATOM    219  CG2 ILE   221      14.842  40.626  65.147  1.00  0.00           C
ATOM    220  CD1 ILE   221      17.793  41.270  64.120  1.00  0.00           C
ATOM    221  H   ILE   221      15.043  44.238  63.130  1.00  0.00           H
ATOM    222  HA  ILE   221      15.645  41.417  62.576  1.00  0.00           H
ATOM    223  HB  ILE   221      14.730  42.660  65.125  1.00  0.00           H
ATOM    224 HG12 ILE   221      17.055  43.198  64.684  1.00  0.00           H
ATOM    225 HG13 ILE   221      17.026  42.219  65.969  1.00  0.00           H
ATOM    226 HG21 ILE   221      15.200  39.781  64.559  1.00  0.00           H
ATOM    227 HG22 ILE   221      15.061  40.553  66.212  1.00  0.00           H
ATOM    228 HG23 ILE   221      13.759  40.742  65.189  1.00  0.00           H
ATOM    229 HD12 ILE   221      17.954  41.684  63.125  1.00  0.00           H
ATOM    230 HD13 ILE   221      18.707  41.306  64.714  1.00  0.00           H
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END
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*UNL residue: inhibitor-01

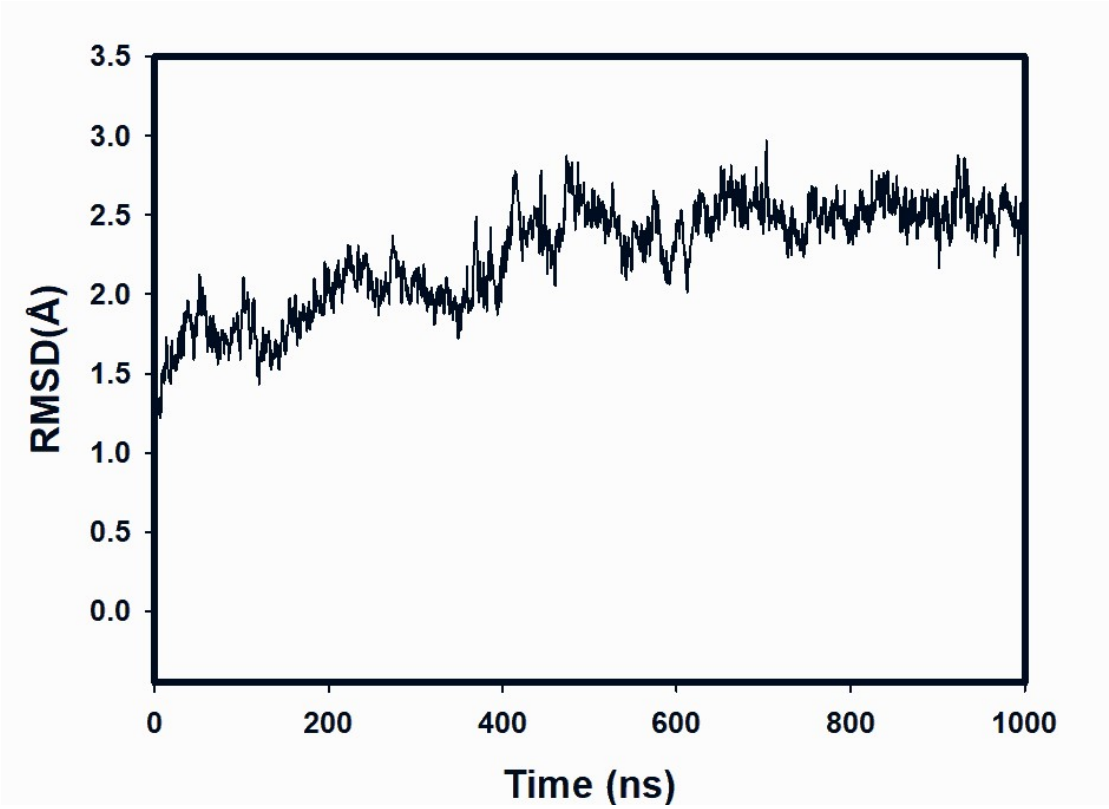

Figure S1. Root-mean-square deviation (RMSD) values of the Zika NS2B-NS3 protease during the 1000 ns production GaMD simulation. The stable RMSD values indicate that the simulation time period is sufficient and reliable for modeling conformational changes in the protein binding pocket.

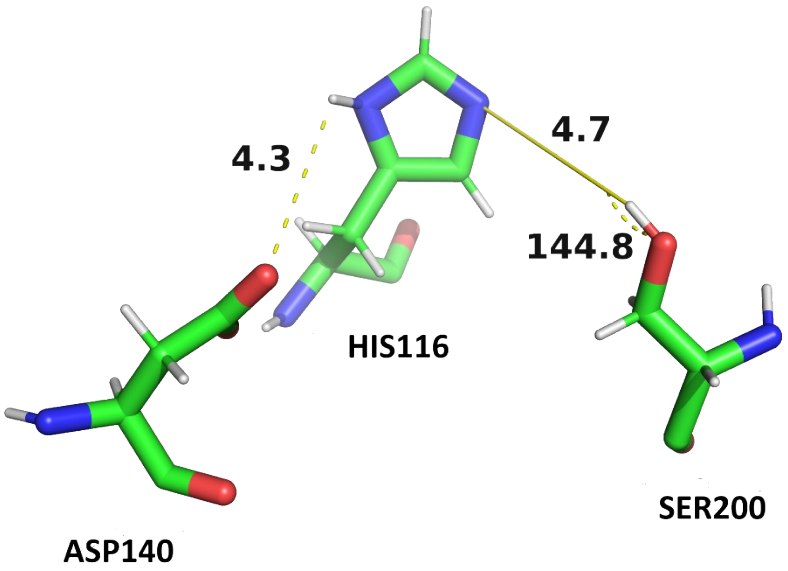

Figure S2. Figure S2. Bond angle and distances of the catalytic triad residues (His116, Asp140, and Ser200) in State I of the Zika NS2B-NS3 protease. The bond angle between Ser O–Ser H···His N is 144.8°, the distance between Ser H···His N is 4.7 Å, and the distance between His H···Asp O is 4.3 Å.

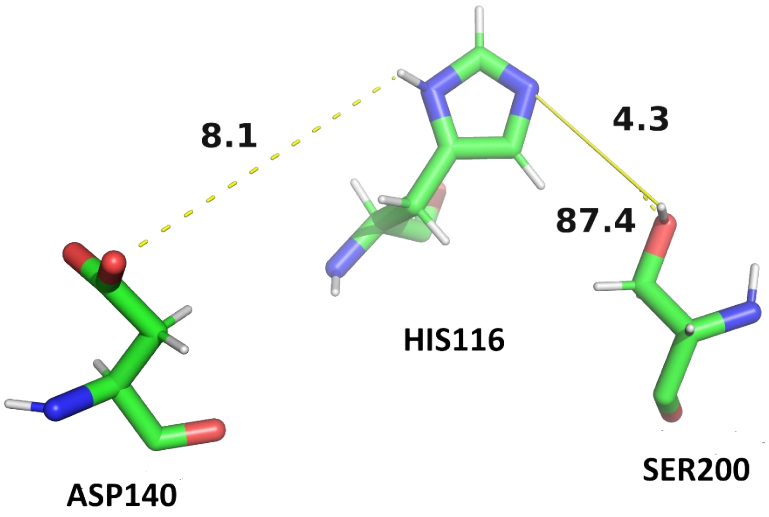

Figure S3. Bond angle and distances of the catalytic triad residues (His116, Asp140, and Ser200) in State II of the Zika NS2B-NS3 protease. The bond angle between Ser O–Ser H···His N is 87.4°, the distance between Ser H···His N is 4.3 Å, and the distance between His H···Asp O is 8.1 Å.