Reposition Pathways of GTP in *Orthoflavivirus* NS5-Methyltransferase Revealed by Enhanced Molecular Dynamics Simulations

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Supplementary Tables and Figures

Table S1: GaMD boost statistics for each independent trajectory.

Replica ID	Average dV (kcal/mol)	Standard Deviation (kcal/mol)
0	86.46702	9.366359
1	88.60070	9.468754
2	88.52820	9.511536
3	88.83112	9.471587
4	89.89656	9.582066
5	89.52380	9.524889
6	89.66230	9.528392
7	91.40238	9.580019
8	88.92563	9.481158
9	88.24013	9.469655
10	90.49797	9.534429
11	90.41958	9.561299
12	89.32847	9.536744
13	88.91878	9.498922
14	89.29779	9.534087
15	88.47932	9.468787
16	90.37966	9.602649
17	88.44150	9.508433
18	90.71362	9.601283
19	89.97502	9.557210

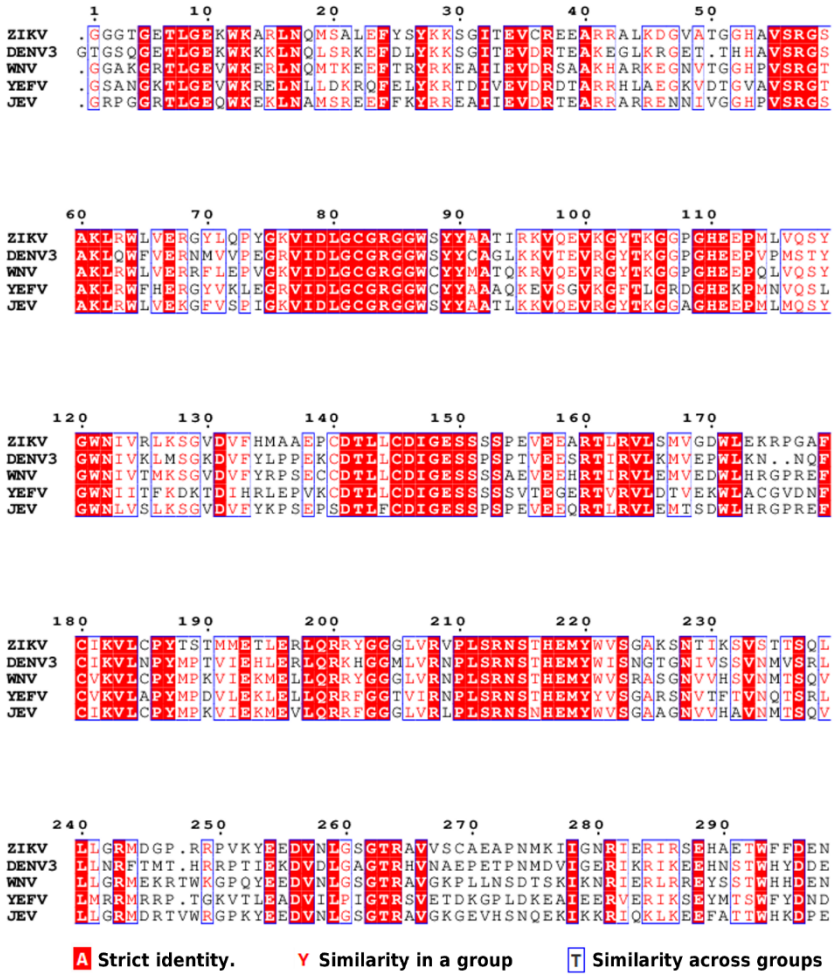

Figure S1: Alignment of MTase sequence from representative *orthoflavivirus*. Identical residues are highlighted in red, similar residues are in blue boxes. Sequence retrieved from UniprotKB,[1] aligned with Clustal Omega[2] and rendered with ESPript 3.0 (https://espript.ibcp.fr).[3]

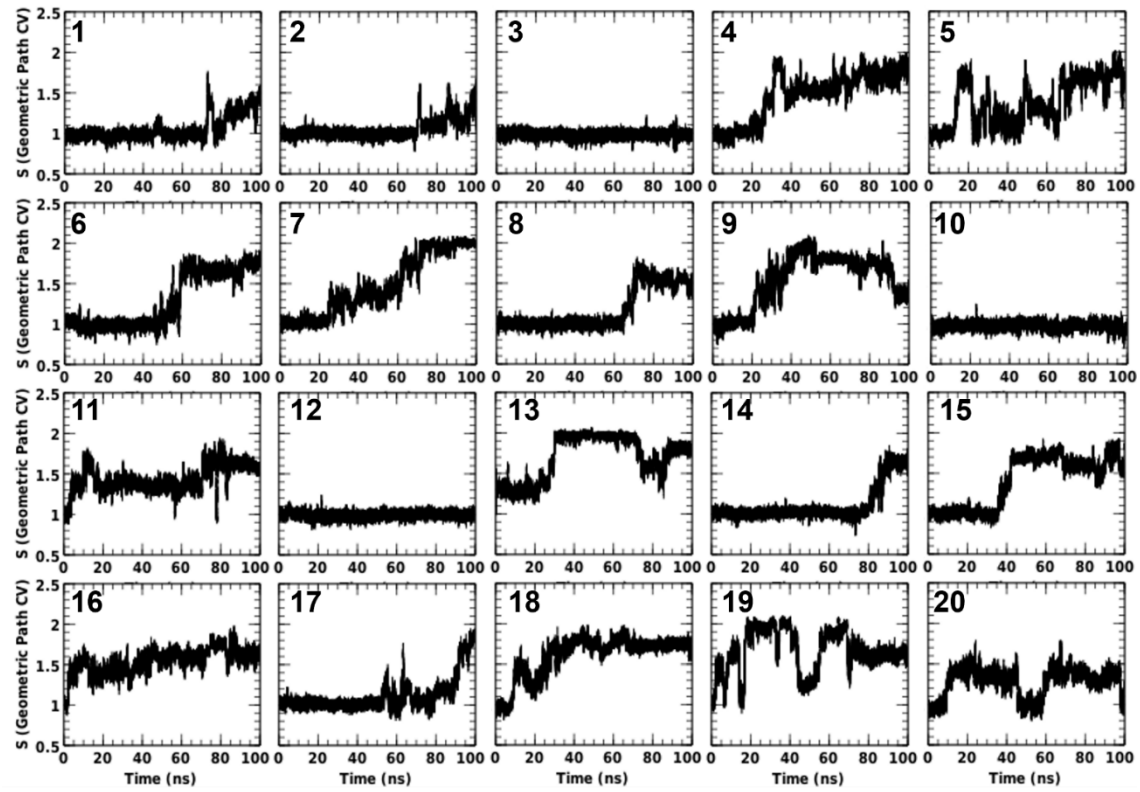

Figure S2. Evolution geometric path CV against simulation time, sampled by the twenty independent GaMD trajectories.

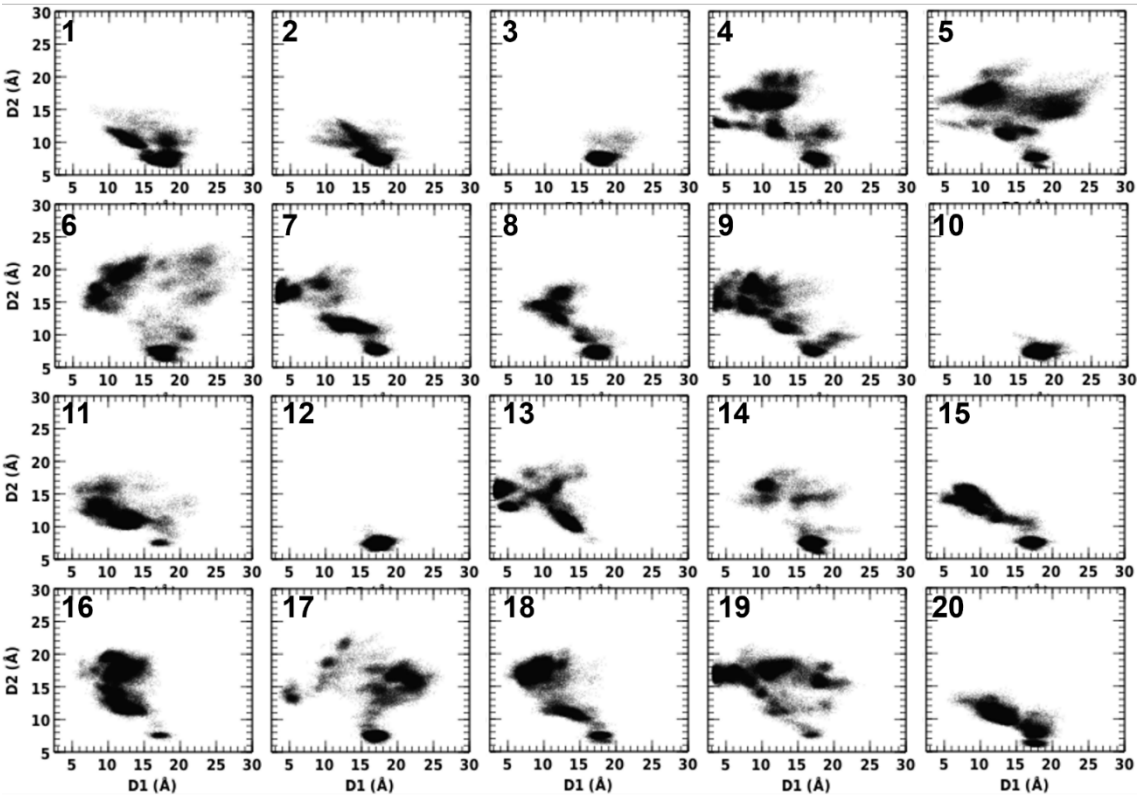

Figure S3: Raw distribution of the two distances being used to define the path CV, sampled by the twenty independent GaMD trajectories. D1 represents the distance between the two substrates of N7-MTase reaction, and D2 represents the distance between the center of mass (COM) of GTP and the experimentally identified GTP-pocket.

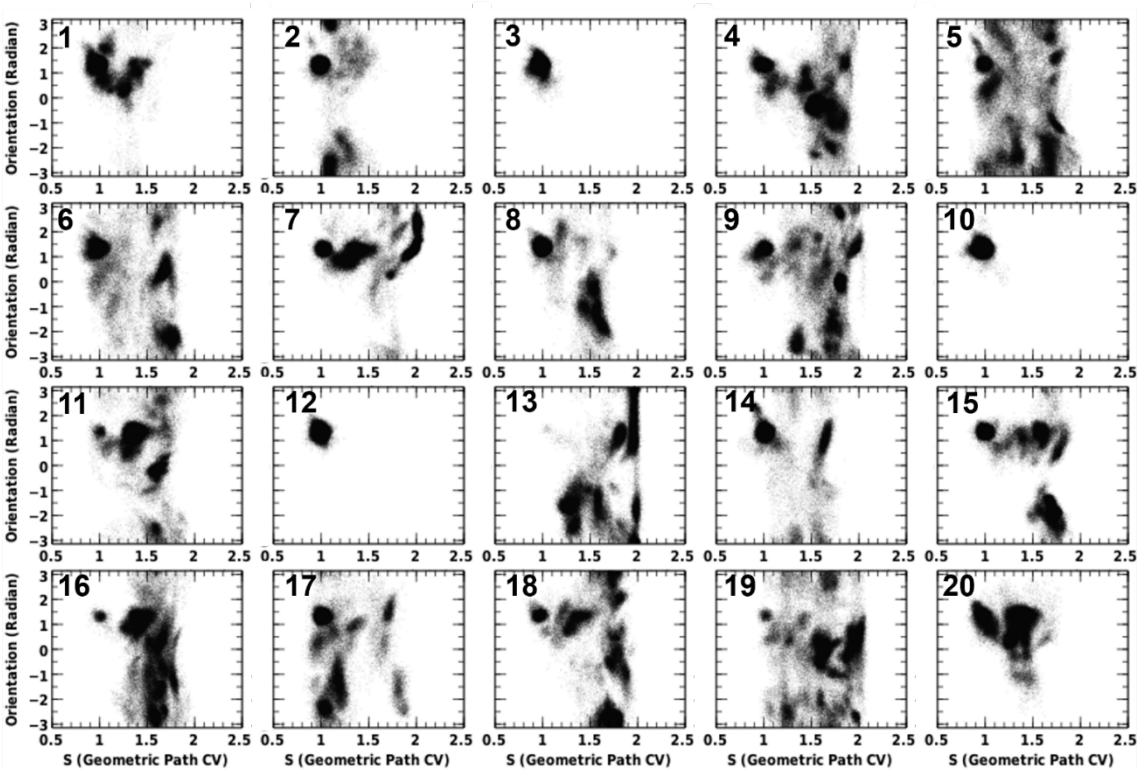

Figure S4: Raw distribution of guanine orientation against geometric path CV, sampled by the twenty independent GaMD trajectories.

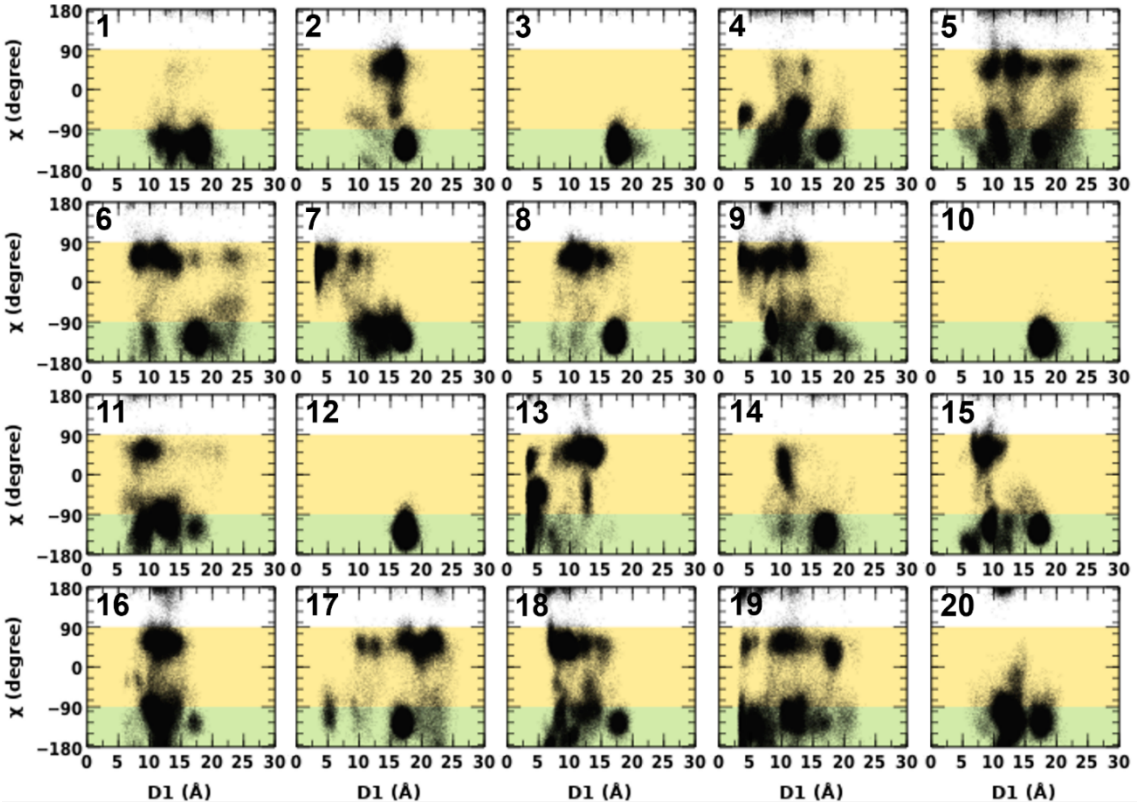

Figure S5: Raw distribution of the χ-angle in guanosine against the distance between atom N7 of GTP and atom CE of SAM, sampled by the twenty independent GaMD trajectories. Regions in χ-angle corresponding to the *syn*-conformation and *anti*-conformation are colored yellow and green, respectively.

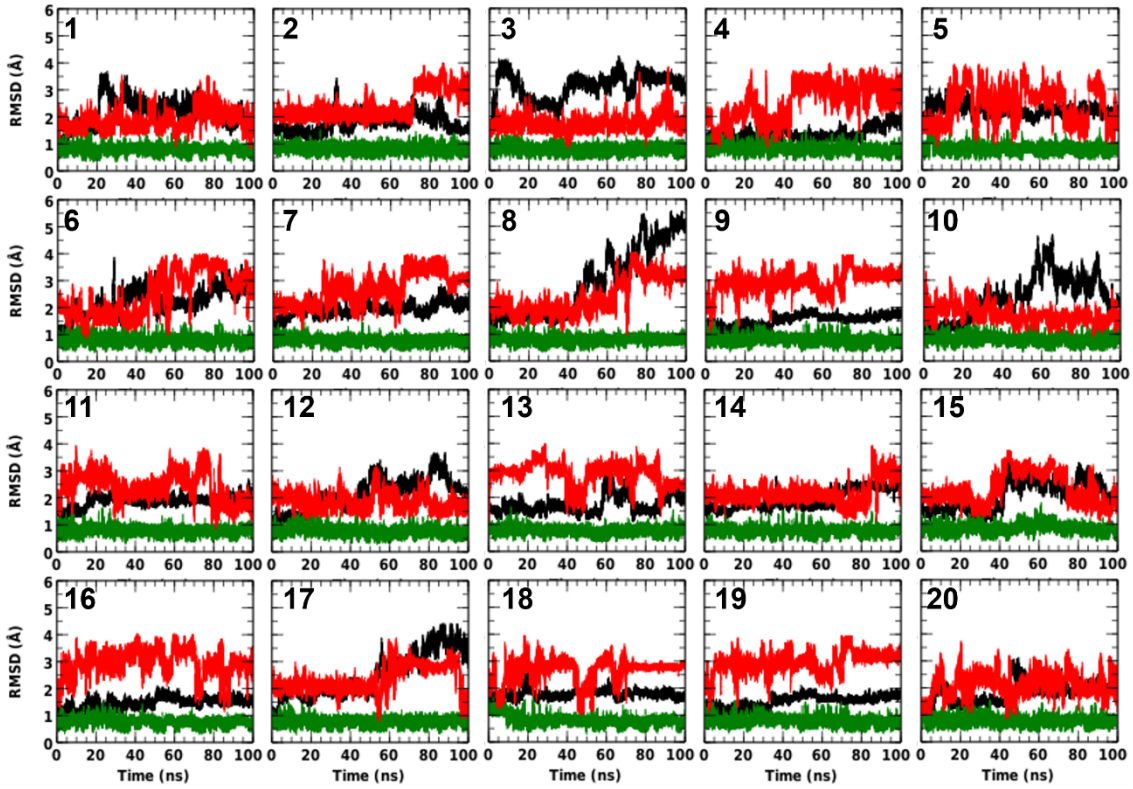

Figure S6. Evolution of fitted RMSD of protein C_α(black), GTP (red) and SAM (green) over the simulation time, sampled by the twenty independent GaMD trajectories.

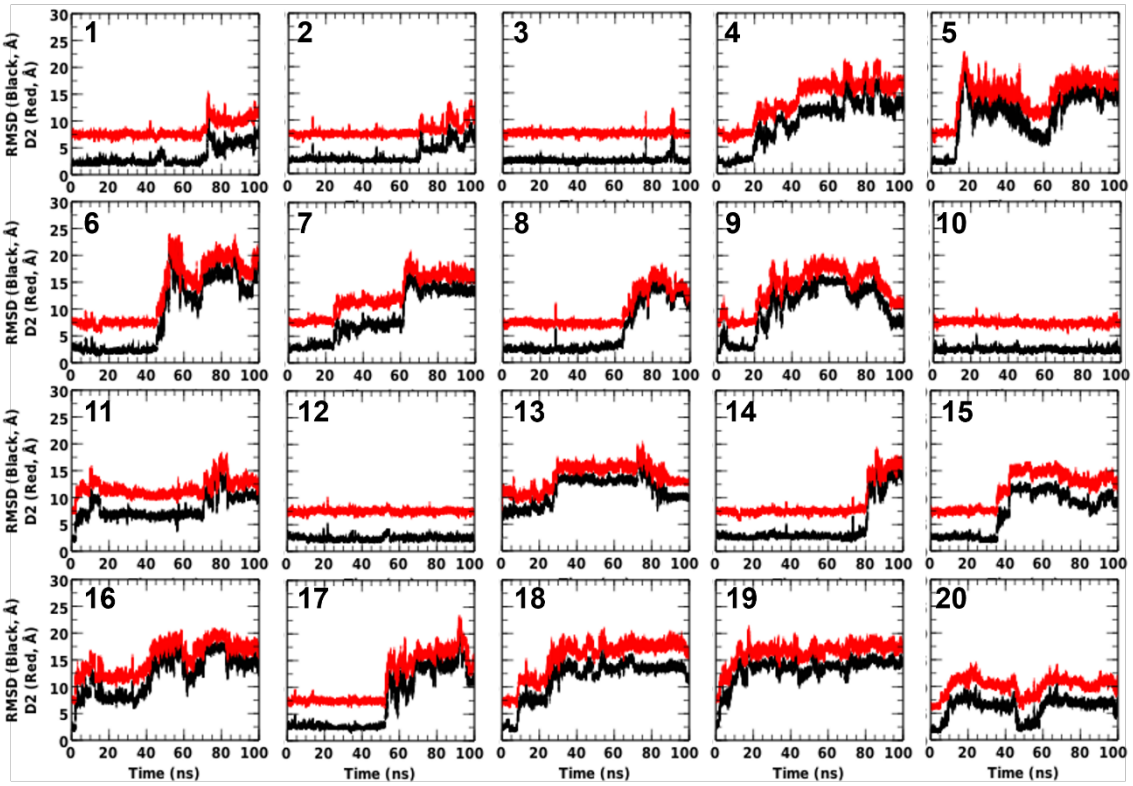

Figure S7. Evolution of D2 (red) and the no-fit RMSD of GTP after RMS fitting of protein backbone (black) over the simulation time, sampled by the twenty independent GaMD trajectories. Similar in shape of the curves suggests that D2 could represent the motion of GTP as it leaves the GTP binding site identified in crystal structures.

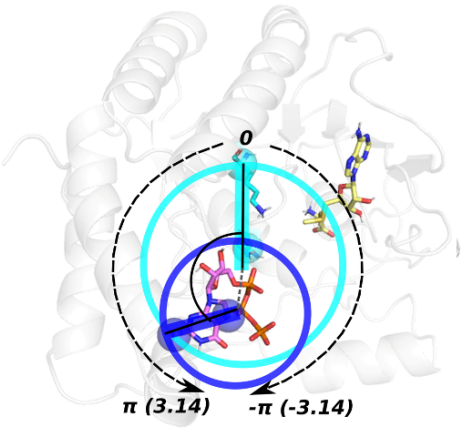

Figure S8. The orientation of GTP is defined by the dihedral angle formed by two atoms on the rigid nucleobase of GTP (exocyclic amino N2 and atom N7 of the guanine base, blue) and two backbone Cα atoms of K61 and K182 (cyan) from the K-D-K-E catalytic tetrad of the MTase protein.

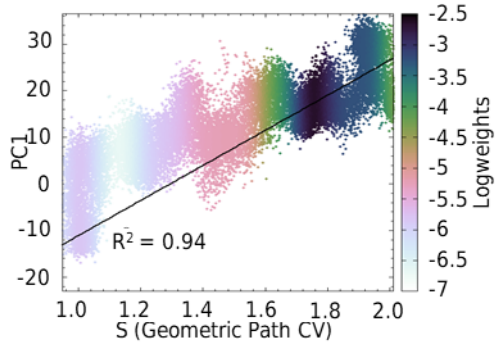

Figure S9. Strong correlation (R^2=0.94) between the MTase global dynamics and the GTP repositioning process along the *syn*-pathway from the weighted linear fitting of PC1 against geometric path CV S. The points in the scatter plot are colored by the log weights calculated from US bias.

Input parameters of MD simulations

1. Input parameters of the final conventional MD equilibration stage before GaMD and the reference conventional MD trials.

```
imin=0,irest=1,ntx=5,
nstlim=${nsteps}, dt=0.002,
ntc=2,ntf=2,
cut=10.0, ntb=2, ntp=1, taup=2.0,
ntpr=10000, ntwx=10000,
ntt=3, gamma_ln=1.0,
temp0=300.0, ig=-1,
```

2. Additional parameters for GaMD trials

   2.1. GaMD equilibration

```
igamd = 3, irest_gamd = 0,
ntcmd = 5720000, nteb = 4550000, ntave = 130000,
ntcmdprep = 520000, ntebprep = 520000,
sigma0P = 6.0, sigma0D = 6.0, iEP = 2, iED=2, iE=2,
```

   2.2. GaMD production

```
igamd = 3, irest_gamd = 1,
ntcmd = 0, nteb = 0, ntave = 130000,
ntcmdprep = 0, ntebprep = 0,
sigma0P = 6.0, sigma0D = 6.0, iEP = 2, iED=2, iE=2,
```

3. Plumed input for umbrella sampling

```
UNITS LENGTH=A ENERGY=kcal/mol
MOLINFO STRUCTURE=reference.pdb

# Orientation
o1: TORSION ATOMS=@N2-266,@N7-266,@CA-59,@CA-180

# Distance of GTP@N7-SAM@CE
d1: DISTANCE ATOMS=@N7-266,@C4-265
# Define COM for pocket and GTP
c1: COM ATOMS=4143-4186
# residues 11,14,15,17,148,213
c2: COM ATOMS=144-165,200-232,250-266,2266-2276,3305-3315
d2: DISTANCE ATOMS=c1,c2
# define path
pp: PATH TYPE=EUCLIDEAN REFERENCE=epath.pdb GPATH NOSPATH NOZPATH
restraint-gspath: RESTRAINT ARG=pp.gspath KAPPA=300.0 AT=${VAR_GSPATH}

PRINT STRIDE=500 ARG=d1,d2,pp.gspath,restraint-gspath.bias,o1 FILE=COLVAR_${RUNID}
```

References

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[2] F. Sievers, A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J. D. Thompson and D. G. Higgins, *Molecular Systems Biology*, 2011, **7**, 539.

[3] X. Robert and P. Gouet, *Nucleic Acids Research*, 2014, **42**, W320–W324.