Effect of mutations on binding of ligands to guanine riboswitch probed by free energy perturbation and molecular dynamics simulations

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File S1. Force field parameters of HPA for Amber

```
%VERSION    VERSION_STAMP = V0001.000    DATE = 06/08/18    09:24:49
%FLAG TITLE
%FORMAT(20a4)
lig
%FLAG POINTERS
%FORMAT(10I8)
         14         7         4        11         8        15        17        23         0         0
         64         1        11        15        23        11        19         9         9         0
          0         0         0         0         0         0         0         0        14         0
          0
%FLAG ATOM_NAME
%FORMAT(20a4)
N1   C2   N3   C4   C5   C6   O6   N7   C8   N9   H1   H2   H3   H4
%FLAG CHARGE
%FORMAT(5E16.8)
  -8.84510442E+00    8.56083654E+00 -1.14563600E+01    1.00823986E+01 -5.98055886E+00
   1.33587681E+01 -1.15438270E+01 -5.04575487E+00    7.93398942E+00 -1.15347159E+01
   6.22291545E+00    1.07147124E+00    5.89855851E+00    1.27738323E+00
%FLAG ATOMIC_NUMBER
%FORMAT(10I8)
         7         6         7         6         6         6         8         7         6         7
         1         1         1         1
%FLAG MASS
%FORMAT(5E16.8)
   1.40100000E+01    1.20100000E+01    1.40100000E+01    1.20100000E+01    1.20100000E+01
   1.20100000E+01    1.60000000E+01    1.40100000E+01    1.20100000E+01    1.40100000E+01
   1.00800000E+00    1.00800000E+00    1.00800000E+00    1.00800000E+00
%FLAG ATOM_TYPE_INDEX
%FORMAT(10I8)
         1         2         3         2         2         2         4         5         2         3
         6         7         6         7
%FLAG NUMBER_EXCLUDED_ATOMS
%FORMAT(10I8)
         9         8         8         9         8         7         2         4         3         2
         1         1         1         1
%FLAG NONBONDED_PARM_INDEX
%FORMAT(10I8)
         1         2         4         7        11        16        22         2         3         5
```

```
         8         12         17         23          4          5          6          9         13         18
        24          7          8          9         10         14         19         25         11         12
        13         14         15         20         26         16         17         18         19         20
        21         27         22         23         24         25         26         27         28
%FLAG RESIDUE_LABEL
%FORMAT(20a4)
HPA
%FLAG RESIDUE_POINTER
%FORMAT(10I8)
         1
%FLAG BOND_FORCE_CONSTANT
%FORMAT(5E16.8)
   3.53830000E+02   3.56210000E+02   5.27310000E+02   4.50710000E+02   4.03490000E+02
   3.69100000E+02   4.16130000E+02   3.54490000E+02   2.95350000E+02   6.52570000E+02
   5.35140000E+02
%FLAG BOND_EQUIL_VALUE
%FORMAT(5E16.8)
   1.38100000E+00   1.37900000E+00   1.01300000E+00   1.31700000E+00   1.08200000E+00
   1.36900000E+00   1.37300000E+00   1.38000000E+00   1.46800000E+00   1.21800000E+00
   1.01000000E+00
%FLAG ANGLE_FORCE_CONSTANT
%FORMAT(5E16.8)
   1.10304000E+02   6.27370000E+01   8.71450000E+01   1.13811000E+02   6.65790000E+01
   4.82850000E+01   7.38710000E+01   6.24610000E+01   9.10570000E+01   1.07603000E+02
   9.26530000E+01   6.71870000E+01   7.04920000E+01   4.71010000E+01   8.67360000E+01
   4.86910000E+01   6.86950000E+01   1.15504000E+02   6.12260000E+01
%FLAG ANGLE_EQUIL_VALUE
%FORMAT(5E16.8)
   2.14675590E+00   2.01934681E+00   1.96698691E+00   2.14762857E+00   2.15146829E+00
   2.08148056E+00   1.84114862E+00   2.19073822E+00   1.94866095E+00   2.19387981E+00
   1.86732857E+00   2.11795796E+00   1.91811767E+00   2.19038915E+00   2.16298747E+00
   2.05163542E+00   1.98679641E+00   1.95860933E+00   2.12144862E+00
%FLAG DIHEDRAL_FORCE_CONSTANT
%FORMAT(5E16.8)
   4.75000000E+00   2.87500000E+00   2.50000000E+00   1.65000000E+00   4.00000000E+00
   1.70000000E+00   2.00000000E+00   1.10000000E+00   1.05000000E+01
%FLAG DIHEDRAL_PERIODICITY
%FORMAT(5E16.8)
   2.00000000E+00   2.00000000E+00   2.00000000E+00   2.00000000E+00   2.00000000E+00
   2.00000000E+00   1.00000000E+00   2.00000000E+00   2.00000000E+00
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```
%FLAG DIHEDRAL_PHASE
%FORMAT(5E16.8)
   3.14159400E+00   3.14159400E+00   3.14159400E+00   3.14159400E+00   3.14159400E+00
   3.14159400E+00   0.00000000E+00   3.14159400E+00   3.14159400E+00
%FLAG SCEE_SCALE_FACTOR
%FORMAT(5E16.8)
   1.20000000E+00   1.20000000E+00   1.20000000E+00   1.20000000E+00   1.20000000E+00
   1.20000000E+00   1.20000000E+00   0.00000000E+00   0.00000000E+00
%FLAG SCNB_SCALE_FACTOR
%FORMAT(5E16.8)
   2.00000000E+00   2.00000000E+00   2.00000000E+00   2.00000000E+00   2.00000000E+00
   2.00000000E+00   2.00000000E+00   0.00000000E+00   0.00000000E+00
%FLAG SOLTY
%FORMAT(5E16.8)
   0.00000000E+00   0.00000000E+00   0.00000000E+00   0.00000000E+00   0.00000000E+00
   0.00000000E+00   0.00000000E+00   0.00000000E+00   0.00000000E+00
%FLAG LENNARD_JONES_ACOEF
%FORMAT(5E16.8)
   7.02078680E+05   7.01106848E+05   6.96551276E+05   7.76656876E+05   7.69642101E+05
   8.49322032E+05   5.15491878E+05   5.17507491E+05   5.74782316E+05   3.76435470E+05
   8.22085792E+05   8.20152428E+05   9.08092643E+05   6.04208667E+05   9.62429652E+05
   1.52360475E+03   1.71458823E+03   2.01475241E+03   9.87857350E+02   1.82491629E+03
   1.34728399E-01   5.06296765E+04   5.22195041E+04   5.87815925E+04   3.59538577E+04
   5.96470418E+04   5.02830284E+01   2.97255425E+03
%FLAG LENNARD_JONES_BCOEF
%FORMAT(5E16.8)
   6.77820248E+02   5.97113873E+02   5.24668528E+02   6.20851625E+02   5.44830041E+02
   5.65406768E+02   5.64804067E+02   4.98871431E+02   5.19385932E+02   4.69350655E+02
   7.75261695E+02   6.82622272E+02   7.09588248E+02   6.46321327E+02   8.86629877E+02
   1.57004457E+01   1.46824710E+01   1.57231024E+01   1.22938581E+01   1.81620725E+01
   7.34107347E-02   1.01949445E+02   9.12729468E+01   9.56653235E+01   8.35449674E+01
   1.16962018E+02   1.59752321E+00   1.38359132E+01
%FLAG BONDS_INC_HYDROGEN
%FORMAT(10I8)
       0      30       3       3      33       5      21      36      11      24
      39       5
%FLAG BONDS_WITHOUT_HYDROGEN
%FORMAT(10I8)
       0       3       1       0      15       2       3       6       4       6
       9       6       9      12       7       9      27       6      12      21
```

```
       8       12       15        9       15       18       10       21       24        8
      24       27        4
%FLAG ANGLES_INC_HYDROGEN
%FORMAT(10I8)
       0        3       33        2        3        0       30        6        6        3
      33        8       12       21       36       14       15        0       30       16
      21       24       39       19       24       21       36       14       27       24
      39        8
%FLAG ANGLES_WITHOUT_HYDROGEN
%FORMAT(10I8)
       0        3        6        1        0       15       12        3        0       15
      18        4        3        0       15        5        3        6        9        7
       6        9       12        9        6        9       27       10        9       12
      21       11        9       12       15       12        9       27       24        7
      12        9       27        9       12       21       24       13       12       15
      18       15       15       12       21       17       21       24       27       18
%FLAG DIHEDRALS_INC_HYDROGEN
%FORMAT(10I8)
      30        0        3        6        4        9        6        3       33        1
       9       12       21       36        6        9       27       24       39        1
      12       21       24       39        6       30        0       15       12        3
      15        0        3       33        4       15       12       21       36        6
      30        0       15       18        7       30        0      -15       18        3
      27       24       21       36        6       30        0        3       33        4
      36       21       24       39        6       30        0       -3      -15        8
      33        0       -3       -6        8       12       24      -21      -36        8
      39       21      -24      -27        8
%FLAG DIHEDRALS_WITHOUT_HYDROGEN
%FORMAT(10I8)
       0        3        6        9        1        0       15      -12        9        2
       0       15       12       21        2        3        0       15       12        3
       3        0       15       18        3        3        6       -9       12        1
       3        6        9       27        1       15        0        3        6        4
       6        9       12       21        5        6        9      -12       15        5
       6        9       27       24        1        9       12      -21       24        6
       9       12       15       18        2        9       27      -24       21        1
      12        9      -27       24        1       12       21      -24       27        6
      15       12        9       27        5       15       12       21       24        6
      18       15       12       21        2       21       12       -9       27        5
      12        6       -9      -27        8       15        9      -12      -21        8
```

```
        12          0       -15        -18          9
%FLAG EXCLUDED_ATOMS_LIST
%FORMAT(10I8)
         2          3          4          5          6          7          8         11         12          3
         4          5          6          7         10         11         12          4          5          6
         8          9         10         11         12          5          6          7          8          9
        10         12         13         14          6          7          8          9         10         11
        13         14          7          8          9         10         11         12         13          8
        11          9         10         13         14         10         13         14         13         14
        12          0         14          0
%FLAG HBOND_ACOEF
%FORMAT(5E16.8)

%FLAG HBOND_BCOEF
%FORMAT(5E16.8)

%FLAG HBCUT
%FORMAT(5E16.8)

%FLAG AMBER_ATOM_TYPE
%FORMAT(20a4)
n   cc  nd  cd  cc  c   o   na  cc  nd  hn  h5  hn  h5
%FLAG TREE_CHAIN_CLASSIFICATION
%FORMAT(20a4)
BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA
%FLAG JOIN_ARRAY
%FORMAT(10I8)
         0          0          0          0          0          0          0          0          0          0
         0          0          0          0
%FLAG IROTAT
%FORMAT(10I8)
         0          0          0          0          0          0          0          0          0          0
         0          0          0          0
%FLAG RADIUS_SET
%FORMAT(1a80)
modified Bondi radii (mbondi)
%FLAG RADII
%FORMAT(5E16.8)
   1.55000000E+00   1.70000000E+00   1.55000000E+00   1.70000000E+00   1.70000000E+00
   1.70000000E+00   1.50000000E+00   1.55000000E+00   1.70000000E+00   1.55000000E+00
```

```
   1.30000000E+00    1.30000000E+00    1.30000000E+00    1.30000000E+00
%FLAG SCREEN
%FORMAT(5E16.8)
   7.90000000E-01    7.20000000E-01    7.90000000E-01    7.20000000E-01    7.20000000E-01
   7.20000000E-01    8.50000000E-01    7.90000000E-01    7.20000000E-01    7.90000000E-01
   8.50000000E-01    8.50000000E-01    8.50000000E-01    8.50000000E-01
%FLAG IPOL
%FORMAT(1I8)
           0
```

File S2. Force field parameters of 6AP for Amber

```
%VERSION    VERSION_STAMP = V0001.000    DATE = 06/13/18    10:13:21
%FLAG TITLE
%FORMAT(20a4)
6AP
%FLAG POINTERS
%FORMAT(10I8)
         17          6          6         12         10         17         18         26          0          0
         76          1         12         17         26         10         18          7          8          0
          0          0          0          0          0          0          0          0         17          0
          0
%FLAG ATOM_NAME
%FORMAT(20a4)
N1    C2    N2    N3    C4    C5    C6    N6    N7    C8    N9    H1    H2    H3    H4    H5    H6
%FLAG CHARGE
%FORMAT(5E16.8)
  -1.53613989E+01    1.67626938E+01 -1.68829610E+01 -1.44867285E+01    8.97994944E+00
  -4.92366546E+00    1.43810392E+01 -1.74296300E+01 -9.71430813E+00    7.06478571E+00
  -7.07025240E+00    7.74083304E+00    7.74083304E+00    8.08705674E+00    8.08705674E+00
    1.29013884E+00    5.75278011E+00
%FLAG ATOMIC_NUMBER
%FORMAT(10I8)
           7          6          7          7          6          6          6          7          7          6
           7          1          1          1          1          1          1
%FLAG MASS
%FORMAT(5E16.8)
   1.40100000E+01    1.20100000E+01    1.40100000E+01    1.40100000E+01    1.20100000E+01
   1.20100000E+01    1.20100000E+01    1.40100000E+01    1.40100000E+01    1.20100000E+01
```

```
   1.40100000E+01   1.00800000E+00   1.00800000E+00   1.00800000E+00   1.00800000E+00
   1.00800000E+00   1.00800000E+00
%FLAG ATOM_TYPE_INDEX
%FORMAT(10I8)
         1         2         3         1         2         2         2         3         1         2
         4         5         5         5         5         6         5
%FLAG NUMBER_EXCLUDED_ATOMS
%FORMAT(10I8)
        12         9         5         9         8         9         6         3         4         3
         2         1         1         1         1         1         1
%FLAG NONBONDED_PARM_INDEX
%FORMAT(10I8)
         1         2         4         7        11        16         2         3         5         8
        12        17         4         5         6         9        13        18         7         8
         9        10        14        19        11        12        13        14        15        20
        16        17        18        19        20        21
%FLAG RESIDUE_LABEL
%FORMAT(20a4)
6AP
%FLAG RESIDUE_POINTER
%FORMAT(10I8)
         1
%FLAG BOND_FORCE_CONSTANT
%FORMAT(5E16.8)
   4.14240000E+02   3.47060000E+02   5.29460000E+02   3.78570000E+02   3.49520000E+02
   3.94620000E+02   4.50710000E+02   3.54490000E+02   4.03680000E+02   5.35140000E+02
%FLAG BOND_EQUIL_VALUE
%FORMAT(5E16.8)
   1.33900000E+00   1.38600000E+00   1.01200000E+00   1.39800000E+00   1.38400000E+00
   1.35200000E+00   1.31700000E+00   1.38000000E+00   1.08200000E+00   1.01000000E+00
%FLAG ANGLE_FORCE_CONSTANT
%FORMAT(5E16.8)
   1.12063000E+02   1.09369000E+02   8.68490000E+01   7.03560000E+01   4.87870000E+01
   1.07575000E+02   6.87670000E+01   8.76350000E+01   6.93770000E+01   4.69790000E+01
   8.71670000E+01   8.61630000E+01   7.46080000E+01   1.15504000E+02   6.24620000E+01
   4.71010000E+01   6.12270000E+01   3.95190000E+01
%FLAG ANGLE_EQUIL_VALUE
%FORMAT(5E16.8)
   2.04098890E+00   2.22110696E+00   2.14570870E+00   2.04587583E+00   2.02580453E+00
   2.21813990E+00   2.09474507E+00   2.08950908E+00   1.97484090E+00   2.19108728E+00
```

2.06542352E+00 2.11097664E+00 1.83050210E+00 1.95860933E+00 2.19073822E+00
2.19038915E+00 2.12144862E+00 2.00922390E+00
%FLAG DIHEDRAL_FORCE_CONSTANT
%FORMAT(5E16.8)
1.05000000E+00 4.80000000E+00 3.62500000E+00 3.00000000E-01 1.70000000E+00
4.75000000E+00 1.10000000E+00
%FLAG DIHEDRAL_PERIODICITY
%FORMAT(5E16.8)
2.00000000E+00 2.00000000E+00 2.00000000E+00 2.00000000E+00 2.00000000E+00
2.00000000E+00 2.00000000E+00
%FLAG DIHEDRAL_PHASE
%FORMAT(5E16.8)
3.14159400E+00 3.14159400E+00 3.14159400E+00 3.14159400E+00 3.14159400E+00
3.14159400E+00 3.14159400E+00
%FLAG SCEE_SCALE_FACTOR
%FORMAT(5E16.8)
1.20000000E+00 1.20000000E+00 1.20000000E+00 1.20000000E+00 1.20000000E+00
1.20000000E+00 0.00000000E+00
%FLAG SCNB_SCALE_FACTOR
%FORMAT(5E16.8)
2.00000000E+00 2.00000000E+00 2.00000000E+00 2.00000000E+00 2.00000000E+00
2.00000000E+00 0.00000000E+00
%FLAG SOLTY
%FORMAT(5E16.8)
0.00000000E+00 0.00000000E+00 0.00000000E+00 0.00000000E+00 0.00000000E+00
0.00000000E+00 0.00000000E+00 0.00000000E+00
%FLAG LENNARD_JONES_ACOEF
%FORMAT(5E16.8)
8.49322032E+05 7.69642101E+05 6.96551276E+05 9.05243612E+05 8.17329084E+05
9.54790629E+05 9.08092643E+05 8.20152428E+05 9.58637891E+05 9.62429652E+05
2.01475241E+03 1.71458823E+03 1.79157166E+03 1.82491629E+03 1.34728399E-01
5.87815925E+04 5.22195041E+04 5.91752946E+04 5.96470418E+04 5.02830284E+01
2.97255425E+03
%FLAG LENNARD_JONES_BCOEF
%FORMAT(5E16.8)
5.65406768E+02 5.44830041E+02 5.24668528E+02 7.17661669E+02 6.90283231E+02
9.06156687E+02 7.09588248E+02 6.82622272E+02 8.96356641E+02 8.86629877E+02
1.57231024E+01 1.46824710E+01 1.82287422E+01 1.81620725E+01 7.34107347E-02
9.56653235E+01 9.12729468E+01 1.18009316E+02 1.16962018E+02 1.59752321E+00
1.38359132E+01

```
%FLAG BONDS_INC_HYDROGEN
%FORMAT(10I8)
       6      33       3       6      36       3      21      39       3      21
      42       3      27      45       9      30      48      10
%FLAG BONDS_WITHOUT_HYDROGEN
%FORMAT(10I8)
       0       3       1       0      18       1       3       6       2       3
       9       1       9      12       1      12      15       4      12      30
       5      15      18       4      15      24       6      18      21       2
      24      27       7      27      30       8
%FLAG ANGLES_INC_HYDROGEN
%FORMAT(10I8)
       3       6      33       5       3       6      36       5      12      30
      48      10      18      21      39       5      18      21      42       5
      24      27      45      15      27      30      48      16      30      27
      45      17      33       6      36      18      39      21      42      18
%FLAG ANGLES_WITHOUT_HYDROGEN
%FORMAT(10I8)
       0       3       6       1       0       3       9       2       0      18
      15       3       0      18      21       1       3       0      18       4
       3       9      12       4       6       3       9       1       9      12
      15       3       9      12      30       6      12      15      18       7
      12      15      24       8      12      30      27       9      15      12
      30      11      15      18      21      12      15      24      27      13
      18      15      24       8      24      27      30      14
%FLAG DIHEDRALS_INC_HYDROGEN
%FORMAT(10I8)
       0       3       6      33       1       0       3       6      36       1
       0      18      21      39       1       0      18      21      42       1
       9       3       6      33       1       9       3       6      36       1
       9      12      30      48       4      12      30      27      45       5
      15      12      30      48       4      15      18      21      39       1
      15      18      21      42       1      15      24      27      45       6
      24      27      30      48       5      45      27      30      48       5
       3      33      -6     -36       7      18      39     -21     -42       7
      45      30     -27     -24       7      12      27     -30     -48       7
%FLAG DIHEDRALS_WITHOUT_HYDROGEN
%FORMAT(10I8)
       0       3       9      12       2       0      18     -15      12       3
       0      18      15      24       3       3       0      18      15       2
```

```
          3         0        18        21         2         3         9       -12        15         2
          3         9        12        30         2        18         0         3         6         2
          6         3         9        12         2        18         0         3         9         2
          9        12       -15        18         3         9        12        15        24         3
          9        12        30        27         4        12        15        18        21         3
         12        15       -24        27         2        12        30       -27        24         5
         15        12       -30        27         4        15        24       -27        30         6
         18        15        12        30         3        18        15        24        27         2
         21        18        15        24         3        24        15       -12        30         3
          0         9        -3        -6         7        15        30       -12        -9         7
         12        18       -15       -24         7        15         0       -18       -21         7
%FLAG EXCLUDED_ATOMS_LIST
%FORMAT(10I8)
          2         3         4         5         6         7         8         9        12        13
         14        15         3         4         5         6         7         8        11        12
         13         4         5         7        12        13         5         6         7         9
         10        11        12        13        17         6         7         8         9        10
         11        16        17         7         8         9        10        11        14        15
         16        17         8         9        10        11        14        15         9        14
         15        10        11        16        17        11        16        17        16        17
         13         0        15         0        17         0
%FLAG HBOND_ACOEF
%FORMAT(5E16.8)

%FLAG HBOND_BCOEF
%FORMAT(5E16.8)

%FLAG HBCUT
%FORMAT(5E16.8)

%FLAG AMBER_ATOM_TYPE
%FORMAT(20a4)
nb   ca   nh   nb   ca   ca   ca   nh   nc   cd   na   hn   hn   hn   hn   h5   hn
%FLAG TREE_CHAIN_CLASSIFICATION
%FORMAT(20a4)
BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA BLA
%FLAG JOIN_ARRAY
%FORMAT(10I8)
          0         0         0         0         0         0         0         0         0         0
          0         0         0         0         0         0         0
```

```
%FLAG IROTAT
%FORMAT(10I8)
          0       0       0       0       0       0       0       0       0       0
          0       0       0       0       0       0       0
%FLAG RADIUS_SET
%FORMAT(1a80)
modified Bondi radii (mbondi)
%FLAG RADII
%FORMAT(5E16.8)
   1.55000000E+00  1.70000000E+00  1.55000000E+00  1.55000000E+00  1.70000000E+00
   1.70000000E+00  1.70000000E+00  1.55000000E+00  1.55000000E+00  1.70000000E+00
   1.55000000E+00  1.30000000E+00  1.30000000E+00  1.30000000E+00  1.30000000E+00
   1.30000000E+00  1.30000000E+00
%FLAG SCREEN
%FORMAT(5E16.8)
   7.90000000E-01  7.20000000E-01  7.90000000E-01  7.90000000E-01  7.20000000E-01
   7.20000000E-01  7.20000000E-01  7.90000000E-01  7.90000000E-01  7.20000000E-01
   7.90000000E-01  8.50000000E-01  8.50000000E-01  8.50000000E-01  8.50000000E-01
   8.50000000E-01  8.50000000E-01
%FLAG IPOL
%FORMAT(1I8)
          0
```

File S3. Force field parameters of HPA for Gromacs:

```
; lig.top created by rdparm2gmx.pl 2018 年  02 月  05 日  星期一  19:51:02 CST

; Include forcefield parameters
#include "amber99sb-ildn.ff/forcefield.itp"

;[ defaults ]
; nbfunc        comb-rule       gen-pairs       fudgeLJ fudgeQQ
;1                  2               yes             0.5     0.8333

[ atomtypes ]
;name   bond_type       mass       charge      ptype          sigma          epsilon
o               o       0.0000    0.0000    A     2.95992e-01    8.78640e-01
n               n       0.0000    0.0000    A     3.25000e-01    7.11280e-01
hn             hn       0.0000    0.0000    A     1.06908e-01    6.56888e-02
```

```
c                c           0.0000   0.0000     A     3.39967e-01    3.59824e-01
cd               cd          0.0000   0.0000     A     3.39967e-01    3.59824e-01
nd               nd          0.0000   0.0000     A     3.25000e-01    7.11280e-01
cc               cc          0.0000   0.0000     A     3.39967e-01    3.59824e-01
na               na          0.0000   0.0000     A     3.25000e-01    7.11280e-01
h5               h5          0.0000   0.0000     A     2.42146e-01    6.27600e-02

[ moleculetype ]
; Name                nrexcl
  HPA                   3

[ atoms ]
;     nr       type  resnr residue  atom   cgnr     charge       mass  typeB    chargeB
      1          n      1     HPA     N1      1    -0.48540   14.000000
      2         cc      1     HPA     C2      2     0.46980   12.000000
      3         nd      1     HPA     N3      3    -0.62870   14.000000
      4         cd      1     HPA     C4      4     0.55330   12.000000
      5         cc      1     HPA     C5      5    -0.32820   12.000000
      6          c      1     HPA     C6      6     0.73310   12.000000
      7          o      1     HPA     O6      7    -0.63350   16.000000
      8         na      1     HPA     N7      8    -0.27690   14.000000
      9         cc      1     HPA     C8      9     0.43540   12.000000
     10         nd      1     HPA     N9     10    -0.63300   14.000000
     11         hn      1     HPA     H1     11     0.34150    1.000000
     12         h5      1     HPA     H2     12     0.05880    1.000000
     13         hn      1     HPA     H3     13     0.32370    1.000000
     14         h5      1     HPA     H4     14     0.07010    1.000000

[ bonds ]
;   ai    aj funct   r     k
    1     11     1   1.0090e-01   3.4326e+05
    2     12     1   1.0790e-01   2.9790e+05
    8     13     1   1.0110e-01   3.4024e+05
    9     14     1   1.0790e-01   2.9790e+05
    1      2     1   1.3800e-01   3.5648e+05
    1      6     1   1.3450e-01   4.0016e+05
    2      3     1   1.3350e-01   4.1388e+05
    3      4     1   1.3760e-01   3.6116e+05
    4     10     1   1.3760e-01   3.6116e+05
    4      5     1   1.3710e-01   4.2175e+05
```

```
      5        8        1   1.3710e-01   3.6719e+05
      5        6        1   1.4620e-01   3.1581e+05
      6        7        1   1.2140e-01   5.4225e+05
      8        9        1   1.3710e-01   3.6719e+05
      9       10        1   1.3350e-01   4.1388e+05

[ pairs ]
;    ai       aj funct
      11        3        1
       4       12        1
       4       14        1
       4       13        1
       5       14        1
      11        5        1
       6       12        1
       6       13        1
      11        7        1
      10       13        1
      11       12        1
      13       14        1
       1        4        1
       1        8        1
       2        5        1
       2        7        1
       2       10        1
       6        3        1
       3        9        1
       3        8        1
       4        7        1
       6       10        1
       6        9        1
       7        8        1

[ angles ]
;    ai       aj       ak funct   theta      cth
       1        2       12        1   1.1561e+02   4.2568e+02
       2        1       11        1   1.1871e+02   4.0233e+02
       3        2       12        1   1.2538e+02   4.1949e+02
       5        8       13        1   1.2566e+02   3.9321e+02
       6        1       11        1   1.1846e+02   4.1179e+02
```

```
     8       9      14       1   1.2210e+02   4.1639e+02
     9       8      13       1   1.2566e+02   3.9321e+02
    10       9      14       1   1.2538e+02   4.1949e+02
     1       2       3       1   1.2271e+02   5.9580e+02
     1       6       5       1   1.1186e+02   5.8735e+02
     1       6       7       1   1.2203e+02   6.3455e+02
     2       1       6       1   1.2419e+02   5.4593e+02
     2       3       4       1   1.0563e+02   5.9488e+02
     3       4      10       1   1.2558e+02   5.8132e+02
     3       4       5       1   1.1168e+02   6.0275e+02
     4      10       9       1   1.0563e+02   5.9488e+02
     4       5       8       1   1.0942e+02   6.1011e+02
     4       5       6       1   1.2151e+02   5.4584e+02
     5       4      10       1   1.1168e+02   6.0275e+02
     5       8       9       1   1.0990e+02   5.7689e+02
     5       6       7       1   1.2571e+02   5.7664e+02
     6       5       8       1   1.1411e+02   5.8565e+02
     8       9      10       1   1.1202e+02   6.2576e+02

[ dihedrals ]
;i    j     k    l      funcC0    ...    C5
     11     1     2      3      3     13.80720      0.00000    -13.80720      0.00000      0.00000      0.00000    ;
     4      3     2      12     3     39.74800      0.00000    -39.74800      0.00000      0.00000      0.00000    ;
     4      10    9      14     3     39.74800      0.00000    -39.74800      0.00000      0.00000      0.00000    ;
     4      5     8      13     3     14.22560      0.00000    -14.22560      0.00000      0.00000      0.00000    ;
     5      8     9      14     3     14.22560      0.00000    -14.22560      0.00000      0.00000      0.00000    ;
     11     1     6      5      3     20.92000      0.00000    -20.92000      0.00000      0.00000      0.00000    ;
     6      1     2      12     3     13.80720      0.00000    -13.80720      0.00000      0.00000      0.00000    ;
     6      5     8      13     3     14.22560      0.00000    -14.22560      0.00000      0.00000      0.00000    ;
     11     1     6      7      3     29.28800     -8.36800    -20.92000      0.00000      0.00000      0.00000    ;
     10     9     8      13     3     14.22560      0.00000    -14.22560      0.00000      0.00000      0.00000    ;
     11     1     2      12     3     13.80720      0.00000    -13.80720      0.00000      0.00000      0.00000    ;
     13     8     9      14     3     14.22560      0.00000    -14.22560      0.00000      0.00000      0.00000    ;
     11     1     2      6      3      9.20480      0.00000     -9.20480      0.00000      0.00000      0.00000    ;
     12     1     2      3      3      9.20480      0.00000     -9.20480      0.00000      0.00000      0.00000    ;
     5      9     8      13     3      9.20480      0.00000     -9.20480      0.00000      0.00000      0.00000    ;
     14     8     9      10     3      9.20480      0.00000     -9.20480      0.00000      0.00000      0.00000    ;
     1      2     3      4      3     39.74800      0.00000    -39.74800      0.00000      0.00000      0.00000    ;
     1      6     5      4      3     24.05800      0.00000    -24.05800      0.00000      0.00000      0.00000    ;
     1      6     5      8      3     24.05800      0.00000    -24.05800      0.00000      0.00000      0.00000    ;
```

```
    2     1     6     5     3     20.92000     0.00000    -20.92000     0.00000     0.00000     0.00000 ;
    2     1     6     7     3     20.92000     0.00000    -20.92000     0.00000     0.00000     0.00000 ;
    2     3     4    10     3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000 ;
    2     3     4     5     3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000 ;
    6     1     2     3     3     13.80720     0.00000    -13.80720     0.00000     0.00000     0.00000 ;
    3     4    10     9     3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000 ;
    3     4     5     8     3     33.47200     0.00000    -33.47200     0.00000     0.00000     0.00000 ;
    3     4     5     6     3     33.47200     0.00000    -33.47200     0.00000     0.00000     0.00000 ;
    4    10     9     8     3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000 ;
    4     5     8     9     3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000 ;
    4     5     6     7     3     24.05800     0.00000    -24.05800     0.00000     0.00000     0.00000 ;
    5     4    10     9     3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000 ;
    5     8     9    10     3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000 ;
    6     5     4    10     3     33.47200     0.00000    -33.47200     0.00000     0.00000     0.00000 ;
    6     5     8     9     3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000 ;
    7     6     5     8     3     24.05800     0.00000    -24.05800     0.00000     0.00000     0.00000 ;
    8     5     4    10     3     33.47200     0.00000    -33.47200     0.00000     0.00000     0.00000 ;
    5     3     4    10     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000 ;
    6     4     5     8     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000 ;
    5     1     6     7     3     87.86400     0.00000    -87.86400     0.00000     0.00000     0.00000 ;


; Include Position restraint file
#ifdef POSRES
#include "posre.itp"
#endif

; Include water topology
#include "amber99sb-ildn.ff/tip3p.itp"

#ifdef POSRES_WATER
; Position restraint for each water oxygen
[ position_restraints ]
;    i funct       fcx        fcy        fcz
     1     1      1000       1000       1000
#endif

; Include topology for ions
#include "amber99sb-ildn.ff/ions.itp"
```

```
[ system ]
14 system in water

[ molecules ]
; Compound              nmols
HPA                       1
SOL               689
NA                        2
CL                        2
```

File S4. Force field parameters of 6AP for Gromacs:

```
; lig.top created by rdparm2gmx.pl 2018 年  02 月  09 日  星期五  06:28:23 CST

; Include forcefield parameters
#include "amber99sb-ildn.ff/forcefield.itp"

;[ defaults ]
; nbfunc              comb-rule            gen-pairs            fudgeLJ fudgeQQ
;1                        2                       yes                    0.5         0.8333

[ atomtypes ]
;name    bond_type       mass        charge      ptype                sigma           epsilon
nd                   nd          0.0000    0.0000    A      3.25000e-01    7.11280e-01
cc                   cc          0.0000    0.0000    A      3.39967e-01    3.59824e-01
nh                   nh          0.0000    0.0000    A      3.25000e-01    7.11280e-01
hn                   hn          0.0000    0.0000    A      1.06908e-01    6.56888e-02
nb                   nb          0.0000    0.0000    A      3.25000e-01    7.11280e-01
na                   na          0.0000    0.0000    A      3.25000e-01    7.11280e-01
ca                   ca          0.0000    0.0000    A      3.39967e-01    3.59824e-01
h5                   h5          0.0000    0.0000    A      2.42146e-01    6.27600e-02

[ moleculetype ]
; Name                nrexcl
   6AP                      3
```

[atoms]

; nr	type	resnr	residue	atom	cgnr	charge	mass	typeB	chargeB
1	na	1	6AP	N9	1	-0.38800	14.000000		
2	cc	1	6AP	C8	2	0.38770	12.000000		
3	nd	1	6AP	N7	3	-0.53310	14.000000		
4	ca	1	6AP	C5	4	-0.27020	12.000000		
5	ca	1	6AP	C4	5	0.49280	12.000000		
6	nb	1	6AP	N3	6	-0.79500	14.000000		
7	ca	1	6AP	C2	7	0.91990	12.000000		
8	nh	1	6AP	N2	8	-0.92650	14.000000		
9	nb	1	6AP	N1	9	-0.84300	14.000000		
10	ca	1	6AP	C6	10	0.78920	12.000000		
11	nh	1	6AP	N6	11	-0.95750	14.000000		
12	hn	1	6AP	H1	12	0.31570	1.000000		
13	h5	1	6AP	H2	13	0.07080	1.000000		
14	hn	1	6AP	H3	14	0.42480	1.000000		
15	hn	1	6AP	H4	15	0.42480	1.000000		
16	hn	1	6AP	H5	16	0.44380	1.000000		
17	hn	1	6AP	H6	17	0.44380	1.000000		

[bonds]

; ai	aj	funct	r	k
1	12	1	1.0110e-01	3.4024e+05
2	13	1	1.0790e-01	2.9790e+05
8	14	1	1.0140e-01	3.3572e+05
8	15	1	1.0140e-01	3.3572e+05
11	16	1	1.0140e-01	3.3572e+05
11	17	1	1.0140e-01	3.3572e+05
1	5	1	1.3500e-01	3.9355e+05
1	2	1	1.3710e-01	3.6719e+05
2	3	1	1.3350e-01	4.1388e+05
3	4	1	1.3360e-01	4.1246e+05
4	5	1	1.3870e-01	4.0033e+05
4	10	1	1.3870e-01	4.0033e+05
5	6	1	1.3420e-01	4.0426e+05
6	7	1	1.3420e-01	4.0426e+05
7	8	1	1.3640e-01	3.7572e+05
7	9	1	1.3420e-01	4.0426e+05
9	10	1	1.3420e-01	4.0426e+05
10	11	1	1.3640e-01	3.7572e+05

```
[ pairs ]
;    ai        aj funct
        12         3         1
         4        13         1
        12         4         1
         4        16         1
         4        17         1
         5        13         1
        12         6         1
         6        14         1
         6        15         1
         9        14         1
         9        15         1
         9        16         1
         9        17         1
        12        13         1
         1        10         1
         1         7         1
         2         6         1
         2        10         1
         3         6         1
         3         9         1
         3        11         1
         4         7         1
         5         9         1
         5        11         1
         5         8         1
         6        10         1
         7        11         1
         8        10         1

[ angles ]
;    ai       aj       ak funct    theta      cth
         1        2        13         1    1.2210e+02    4.1639e+02
         2        1        12         1    1.2566e+02    3.9321e+02
         3        2        13         1    1.2538e+02    4.1949e+02
         5        1        12         1    1.2559e+02    3.9857e+02
         7        8        14         1    1.1613e+02    4.1070e+02
         7        8        15         1    1.1613e+02    4.1070e+02
```

```
    10     11     16      1   1.1613e+02   4.1070e+02
    10     11     17      1   1.1613e+02   4.1070e+02
    14      8     15      1   1.1485e+02   3.3514e+02
    16     11     17      1   1.1485e+02   3.3514e+02
     1      5      4      1   1.1834e+02   5.8752e+02
     1      5      6      1   1.2707e+02   5.9078e+02
     1      2      3      1   1.1202e+02   6.2576e+02
     2      1      5      1   1.1315e+02   5.7287e+02
     2      3      4      1   1.0547e+02   6.0450e+02
     3      4      5      1   1.1972e+02   5.8693e+02
     3      4     10      1   1.1972e+02   5.8693e+02
     4      5      6      1   1.2263e+02   5.7873e+02
     4     10      9      1   1.2263e+02   5.7873e+02
     4     10     11      1   1.2013e+02   5.8024e+02
     5      4     10      1   1.1997e+02   5.6216e+02
     5      6      7      1   1.1586e+02   5.7396e+02
     6      7      8      1   1.1695e+02   6.1254e+02
     6      7      9      1   1.2719e+02   5.9229e+02
     7      9     10      1   1.1586e+02   5.7396e+02
     8      7      9      1   1.1695e+02   6.1254e+02
     9     10     11      1   1.1695e+02   6.1254e+02

[ dihedrals ]
;i    j     k    l      funcC0    ...    C5
    12     1     2     3      3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000  ;
     4     3     2    13      3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000  ;
    12     1     5     4      3      2.51040     0.00000     -2.51040     0.00000     0.00000     0.00000  ;
     4    10    11    16      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     4    10    11    17      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     5     1     2    13      3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000  ;
    12     1     5     6      3      2.51040     0.00000     -2.51040     0.00000     0.00000     0.00000  ;
     6     7     8    14      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     6     7     8    15      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     9     7     8    14      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     9     7     8    15      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     9    10    11    16      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
     9    10    11    17      3      8.78640     0.00000     -8.78640     0.00000     0.00000     0.00000  ;
    12     1     2    13      3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000  ;
    12     1     2     5      3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000  ;
    13     1     2     3      3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000  ;
```

```
     7     14     8     15     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000   ;
    10     16    11     17     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000   ;
     1      5     4      3     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     1      5     4     10     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     1      5     6      7     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     1      2     3      4     3     39.74800     0.00000    -39.74800     0.00000     0.00000     0.00000   ;
     2      1     5      4     3      2.51040     0.00000     -2.51040     0.00000     0.00000     0.00000   ;
     2      1     5      6     3      2.51040     0.00000     -2.51040     0.00000     0.00000     0.00000   ;
     2      3     4      5     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     2      3     4     10     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     5      1     2      3     3     14.22560     0.00000    -14.22560     0.00000     0.00000     0.00000   ;
     3      4     5      6     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     3      4    10      9     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     3      4    10     11     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     4      5     6      7     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     4     10     9      7     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     5      4    10      9     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     5      4    10     11     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     5      6     7      8     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     5      6     7      9     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     6      5     4     10     3     30.33400     0.00000    -30.33400     0.00000     0.00000     0.00000   ;
     6      7     9     10     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     7      9    10     11     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     8      7     9     10     3     40.16640     0.00000    -40.16640     0.00000     0.00000     0.00000   ;
     5     10     4      3     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000   ;
     4      1     5      6     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000   ;
     6      9     7      8     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000   ;
     4      9    10     11     3      9.20480     0.00000     -9.20480     0.00000     0.00000     0.00000   ;

; Include Position restraint file
#ifdef POSRES
#include "posre.itp"
#endif

; Include water topology
#include "amber99sb-ildn.ff/tip3p.itp"

#ifdef POSRES_WATER
; Position restraint for each water oxygen
[ position_restraints ]
```

```
;    i funct            fcx              fcy              fcz
      1       1          1000             1000             1000
#endif

; Include topology for ions
#include "amber99sb-ildn.ff/ions.itp"

[ system ]
17 system in water

[ molecules ]
; Compound              nmols
6AP                       1
SOL                 776
NA                        2
CL                        2
```

Table S1. The parameters of the simulated systems produced by the Leap module in Amber[a]

Ligands	RNA	Box size (Å)	Atom number of system	Counter ions(Na+)
HPA	WT	92.736×70.287×60.137	34937	66
	A24U	89.960×70.742×59.445	33536	66
	U25A/A46G	90.120×71.1985×9.803	34137	66
	A24U/U25A/A46G	90.026×71.734×59.215	34080	66
6AP	WT	92.736×70.287×60.137	34940	66
	U25A/A46G/C74U	89.894×71.278×58.972	33677	66
	A24U/U25A/A46G/C74U	90.047×72.266×63.302	36566	66

[a]All information were automatically produced by the Leap module in Amber by setting the parameters described in our manuscript.

Table S2. Hydrogen bonding interactions of HPA with key residues in the WT and mutated GRs

[a]Hydrogen bonds	WT GR		A24U		U25A/A46G		A24U/U25A/A46G	
	[b]Occupancy (%)	Distance (Å)	Occupancy (%)	Distance (Å)	Occupancy (%)	Distance (Å)	Occupancy (%)	Distance (Å)
[c]HPA@O6...C74@N4...H41	73.92	2.98	[d] --	--	92.85	2.94	94.46	2.95
HPA@N3...U51@N3...H3	58.24	3.00	84.86	3.01	45.14	3.11	97.58	29.7
HPA@O6...U22@O2′-HO2′	--	--	33.32	2.81	--	--	--	--
U22@O2′...HPA@N7-H3	80.17	2.93	95.44	2.89	91.22	2.91	91.24	2.96
C74@N3...HPA@N1-H1	78.84	2.89	--	--	93.17	2.91	95.17	2.95
C74@O2...HPA@N1-H1	--	--	98.58	2.96	--	--	--	--

[a]Hydrogen bonds are determined by the acceptor···donor distance of < 3.5Å and acceptor···H-donor angle of > 120°.
[b]Occupancy(%) is defined as the percentage of simulation time that a specific hydrogen bond exists.
[c]The full lines indicate chemical bonds, and the dotted lines describe hydrogen bonding interactions.
[d]The symbol "--" indicates that occupancy of hydrogen bonds formed between HPA and nucleotides is lower than 20%.

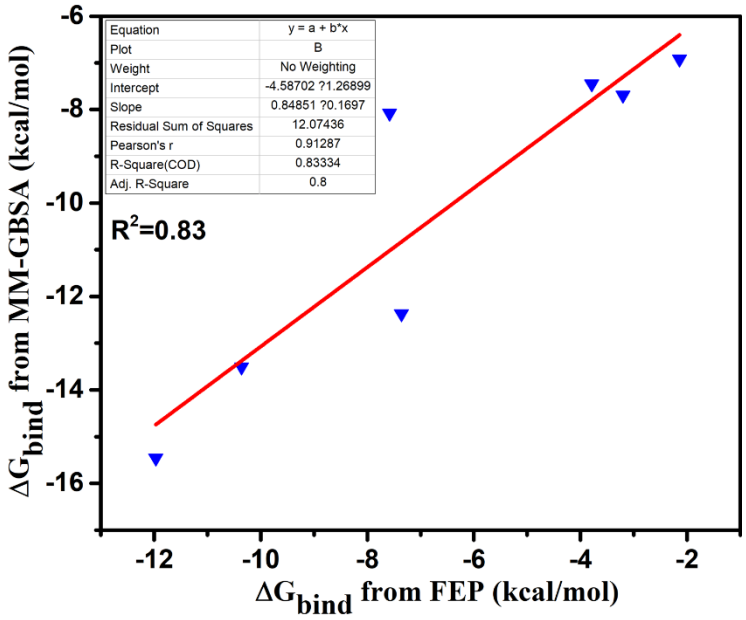

Figure S1. Correlation relationship between binding free energies calculated by FEP method and that by MM-GBSA method.

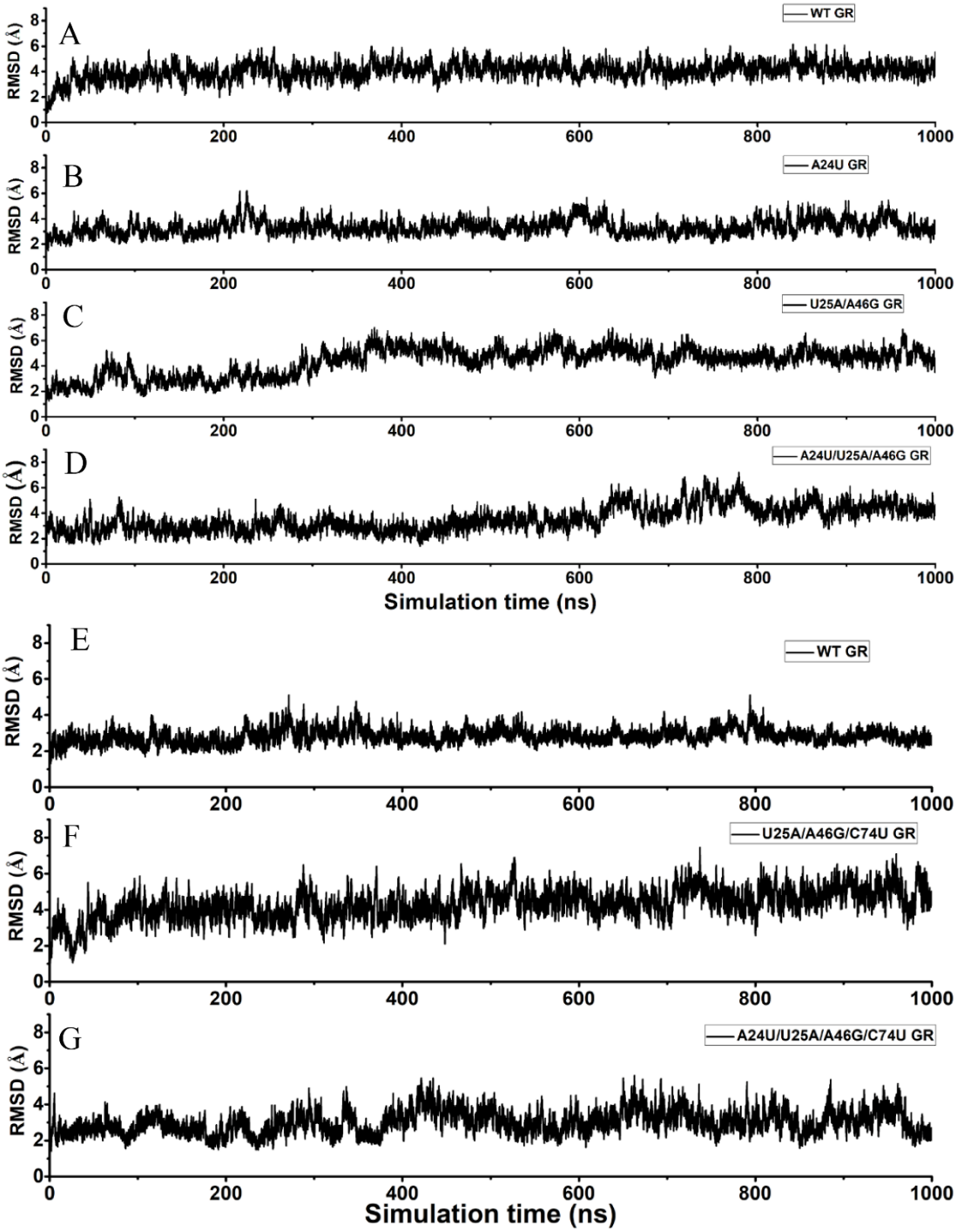

Figure S2. Root-mean-square deviations (RMSDs) of atoms P1, O3′, O5′, C3′, C4′ and C5′ in GR: (A)-(D) the WT and mutated GRs with ligand HPA, (E)-(G) the WT and mutated GRs with ligand 6AP.

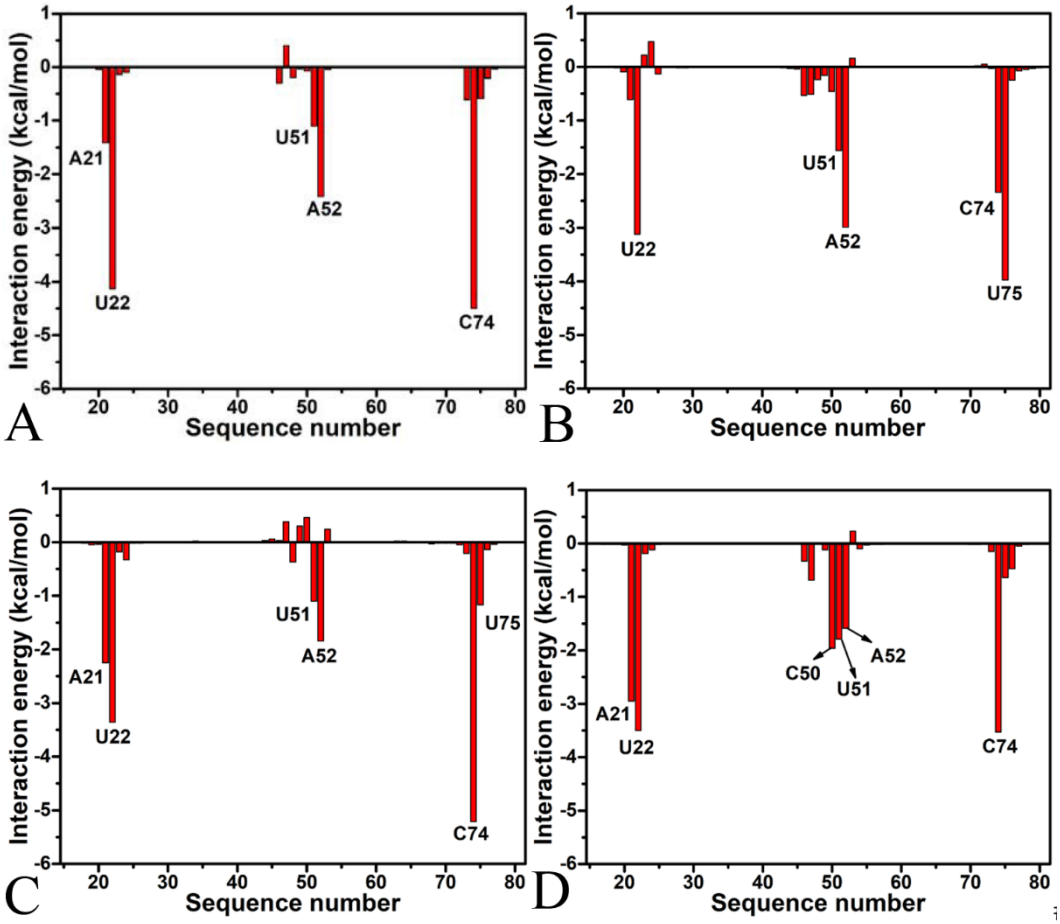

Figure S3. HPA-nucleotide interaction spectrum: (A) the WT GR, (B) the GR with mutation A24U, (C) the GR with mutations U25A/A46G and (D) the GR with mutations A24U/U25A/A46G. Only nucleotides of interaction energies stronger than 1.0 kcal/mol were labeled.

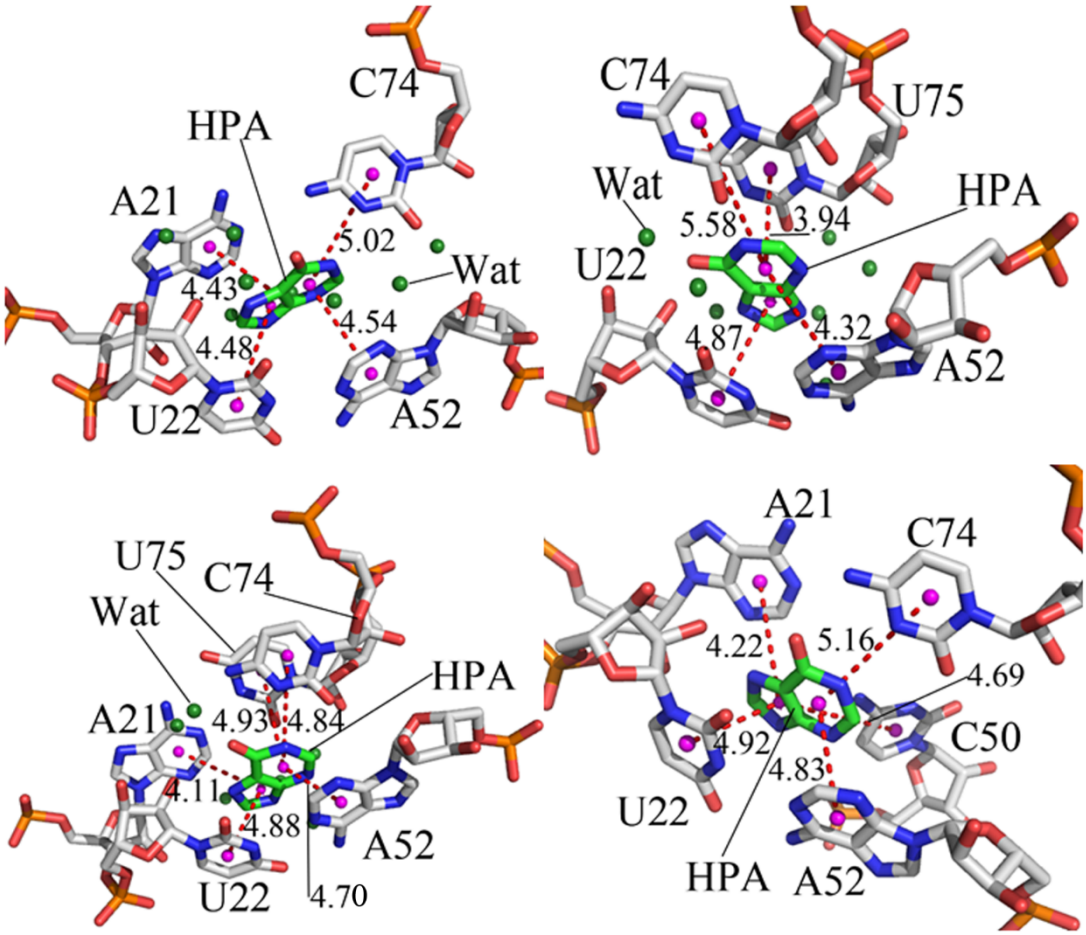

Figure S4. Hydrophobic interactions of HPA with key nucleotides in the WT and mutated GRs: (A) WT, (B) A24U, (C) U25A/A46G and (D) A24U/U25A/A46G. The key nucleotides and HPA are shown in stick modes and water molecules in the forest color balls. The magenta balls represent pseudoatoms forming hydrophobic interactions between 6AP and nucleotides.

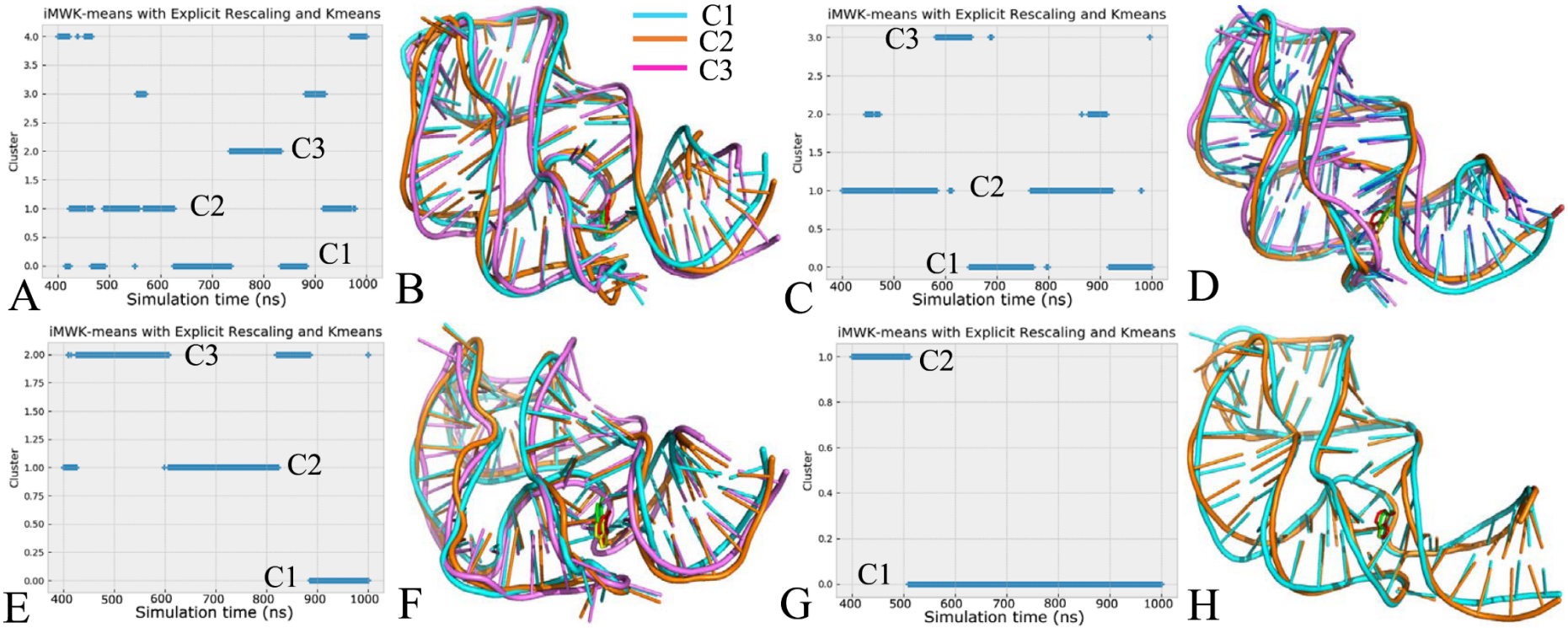

Figure S5. Cluster analyses and superimpositions of corresponding structures: (A), (C), (E) and (G) depict the cluster analyses performed by using iMWK-Means method, and (A), (C), (E) and (G) respectively represent the WT, A24U, U25A/A46G and A24U/U25A/A46G GRs complexed with HPA; (B), (D), (F) and (H) display superimpositions of structures extracted from different clusters C1, C2 and C3. GRs are shown in cartoon modes and HPA is displayed in stick modes with green, red and yellow.

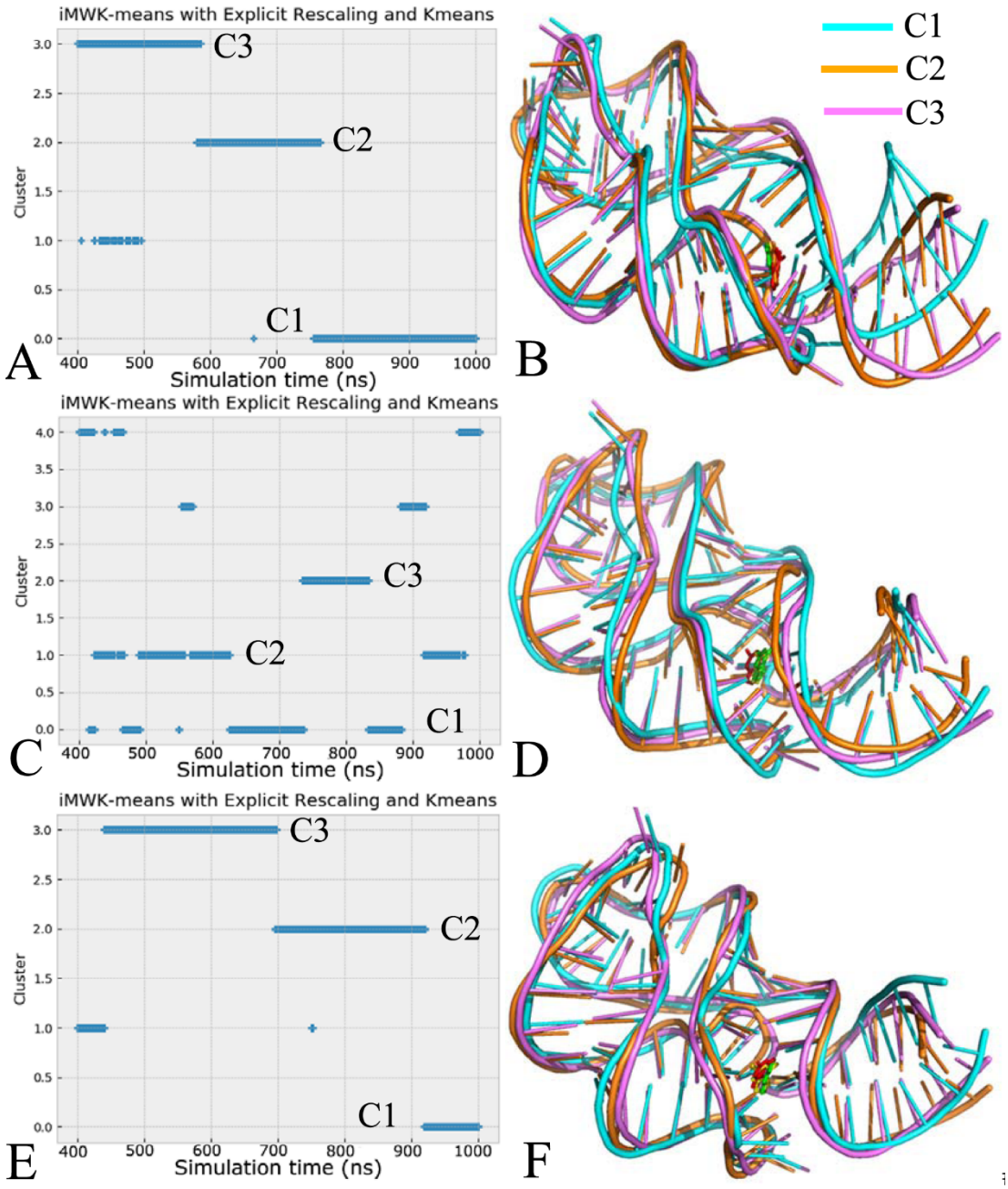

Figure S6. Cluster analyses and superimpositions of corresponding structures: (A), (C) and (E) depict the cluster analyses performed by using iMWK-Means method, and (A), (C) and (E) respectively indicate the WT, U25A/A46G/C74U and A24U/U25A/A46G/C74U GRs complexed with 6AP; (B), (D)and (F) show superimpositions of structures extracted from different clusters C1, C2 and C3. GRs are depicted in cartoon modes and 6AP is shown in stick modes with green, red and yellow.

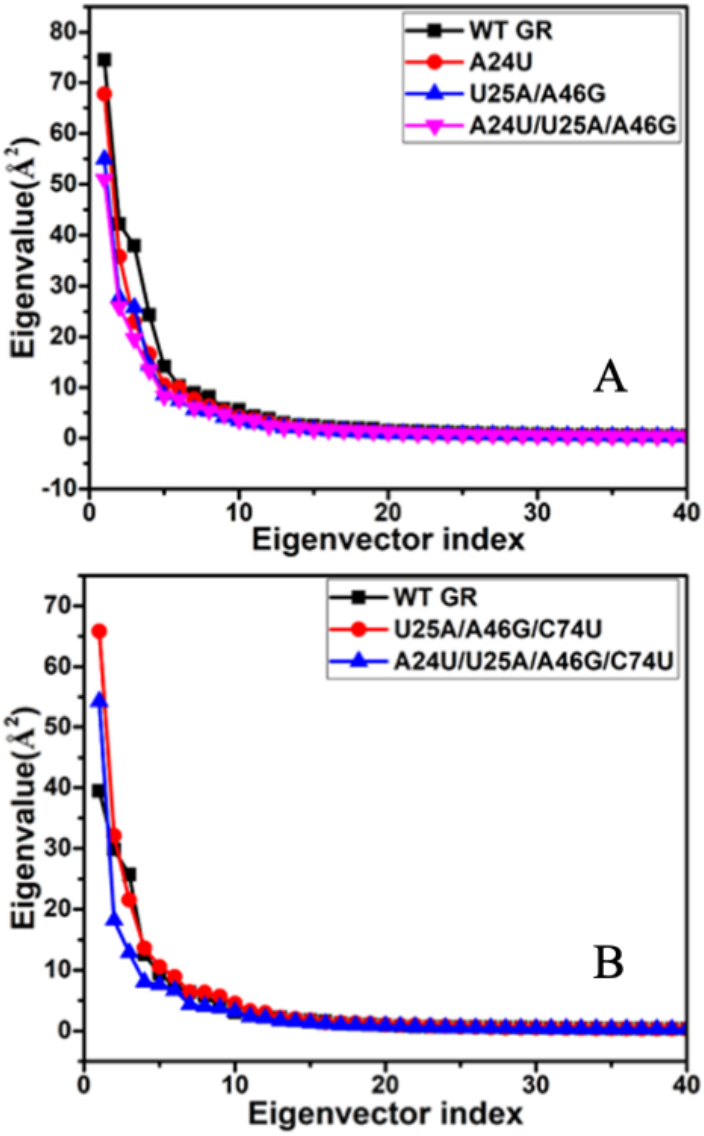

Figure S7. Comparison of the eigenvalues plotted against the corresponding eigenvector indices obtained from the covariance matrix of C1′ atoms in GR constructed from the equilibrated MD trajectories: (A) the WT and mutated GRs complexed with HPA and (B) the WT and mutated GRs complexed with 6AP.

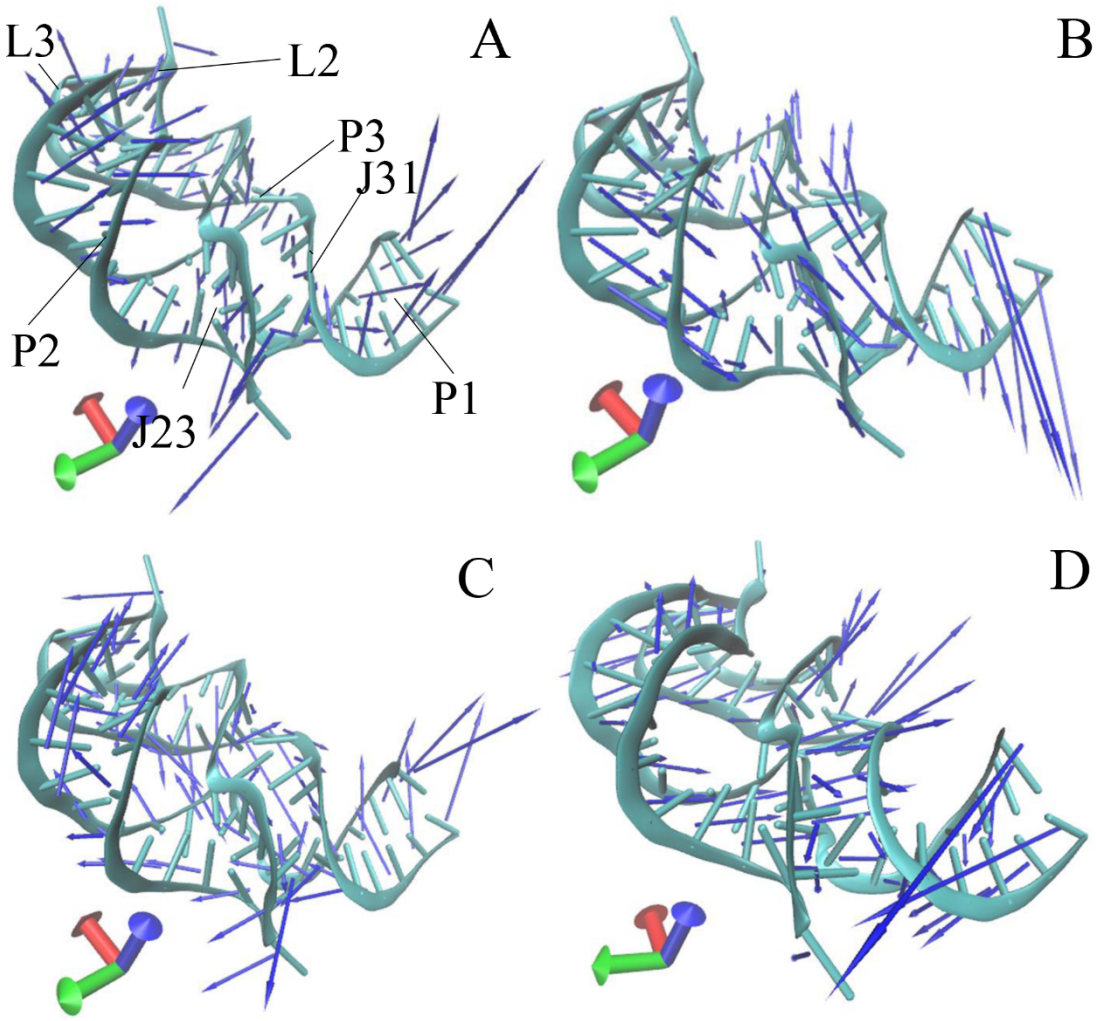

Figure S8. Collective motions reflected by the first eigenvector corresponding to the principal component PC1 obtained by performing principal component analysis (PCA) on the equilibrated MD trajectories of the WT and mutated GRs complexed with HPA: (A) WT, (B) A24U, (C) U25A/A46G and (D) A24U/U25A/A46G. In this figure, the direction and length of the arrows respectively reflect the motion direction and strength of nucleotides in GR.

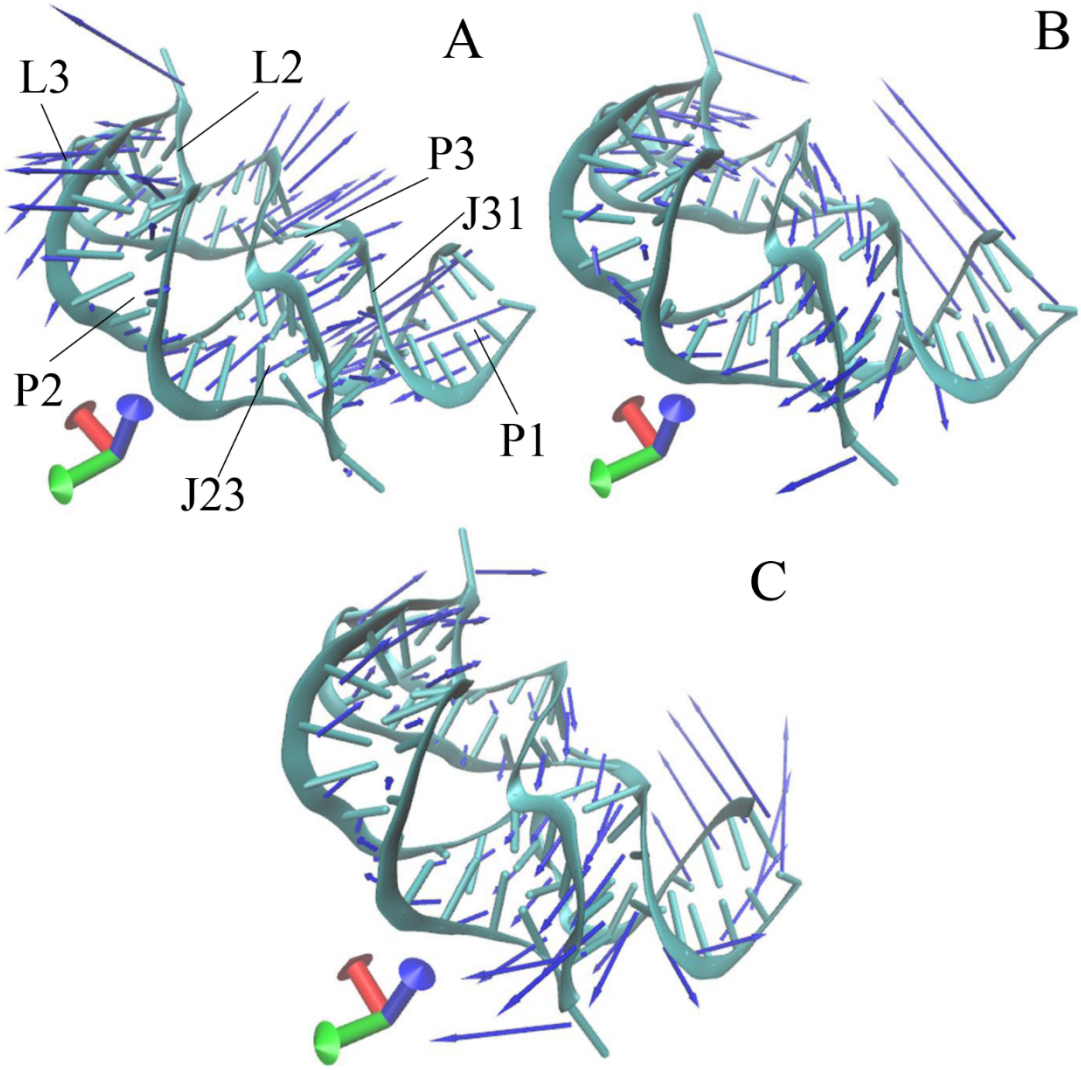

Figure S9. Collective motions reflected by the first eigenvector corresponding to the principal component PC1 obtained by performing principal component analysis (PCA) on the equilibrated MD trajectories of the WT and mutated GRs complexed with 6AP: (A) WT, (B) U25A/A46G/C74U and (C) A24U/U25A/A46G/C74U. In this figure, the direction and length of the arrows respectively reflect the motion direction and strength of nucleotides in GR.

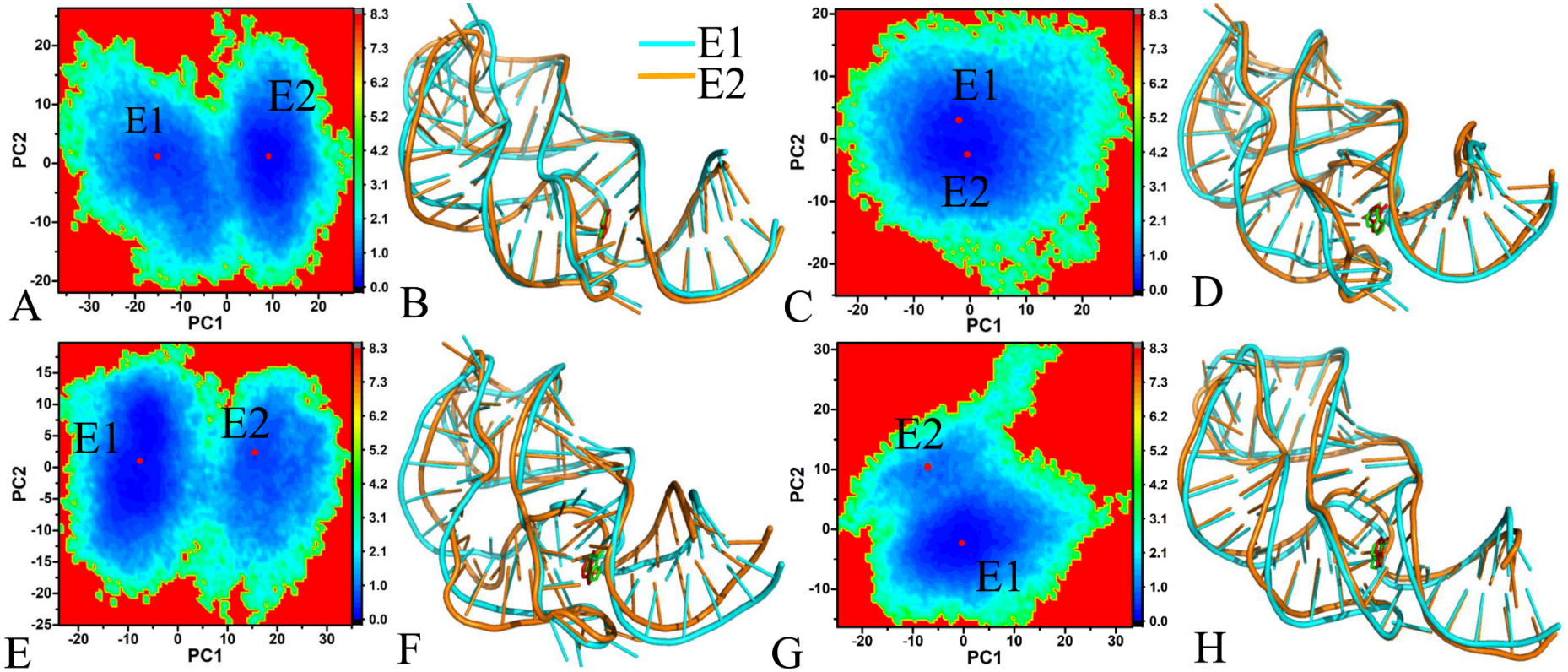

Figure S10. Free energy landscapes and structural superimposition of the WT and mutated GRs with HPA: (A), (C), (E) and (G) depict free energy landscapes constructed using projection of MD trajectories on the first two principal components PC1 and PC2 from the diagonalization of covariance matrix, among which (A), (C), (E) and (G) separately correspond to the WT, A24U, U25A/A46G and A24U/U25A/A46G GRs; (B), (D), (F) and (H) display superimpositions of structures represented by free energy basins E1 and E2, and (B), (D), (F) and (H) respectively indicate the WT, A24U, U25A/A46G and A24U/U25A/A46G GRs. GRs are shown in cartoon modes and HPA is displayed in stick modes with green, red and yellow.

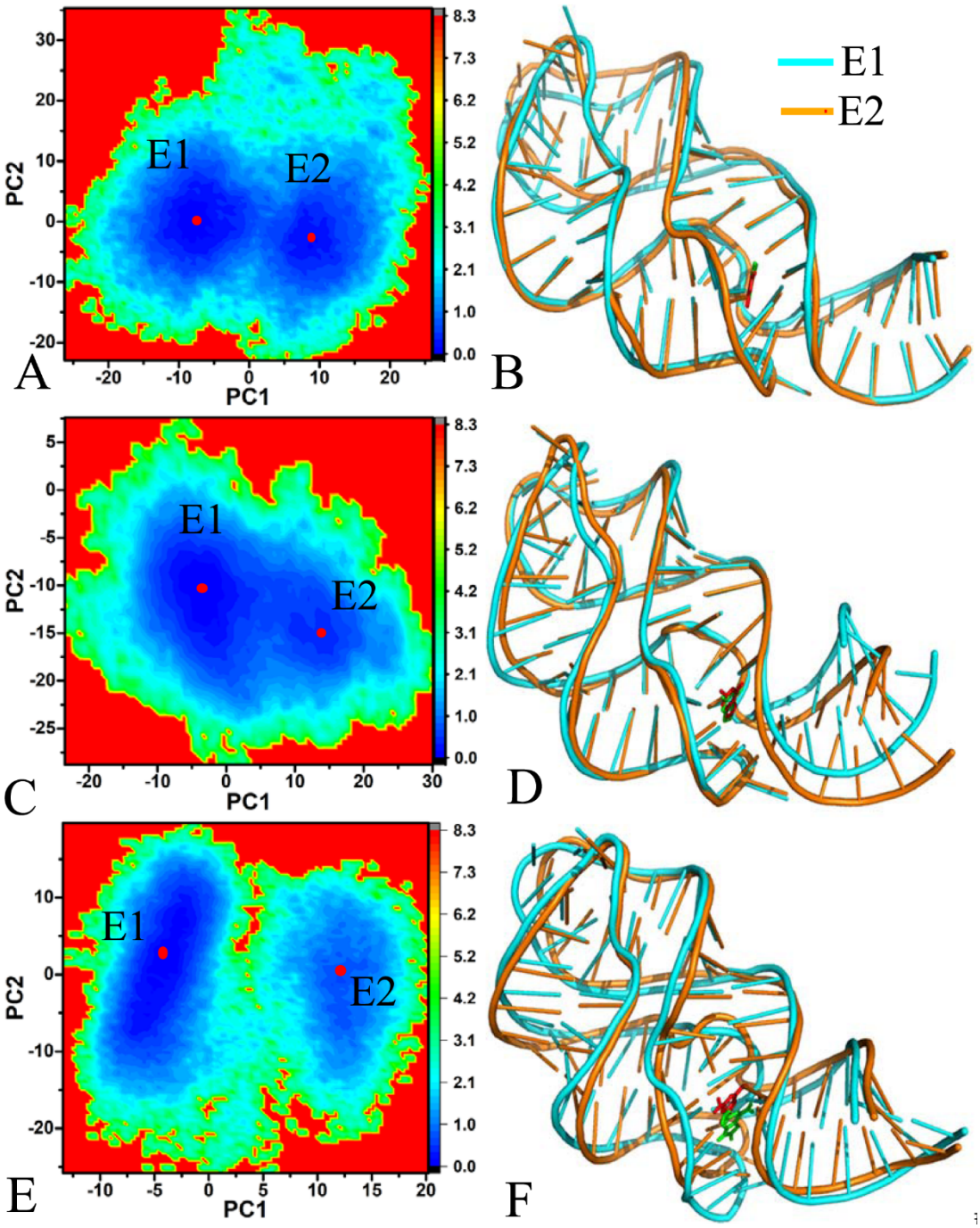

Figure S11. Free energy landscapes and structural superimposition of the WT and mutated GRs with 6AP: (A), (C) and (E) depict free energy landscapes constructed using projection of MD trajectories on the first two principal components PC1 and PC2 from the diagonalization of covariance matrix, among which (A), (C) and (E) separately correspond to the WT, U25A/A46G/C74U and A24U/U25A/A46G/C74U GRs; (B), (D) and (F) display superimpositions of structures represented by free energy basins E1 and E2, and (B), (D) and (F) respectively indicate the WT, U25A/A46G/C74U and A24U/U25A/A46G/C74U GRs. GRs are shown in cartoon modes and 6AP is displayed in stick modes with green, red and yellow