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Supporting Material

Long Timescale Molecular Dynamics Simulations of the Major Urinary Protein Provide Atomistic Interpretations for the Unusual Thermodynamics of Ligand Binding

Julie Roy and Charles A. Laughton

Supplementary material for:

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*Julie Roy and Charles A Laughton**

School of Pharmacy and Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK.

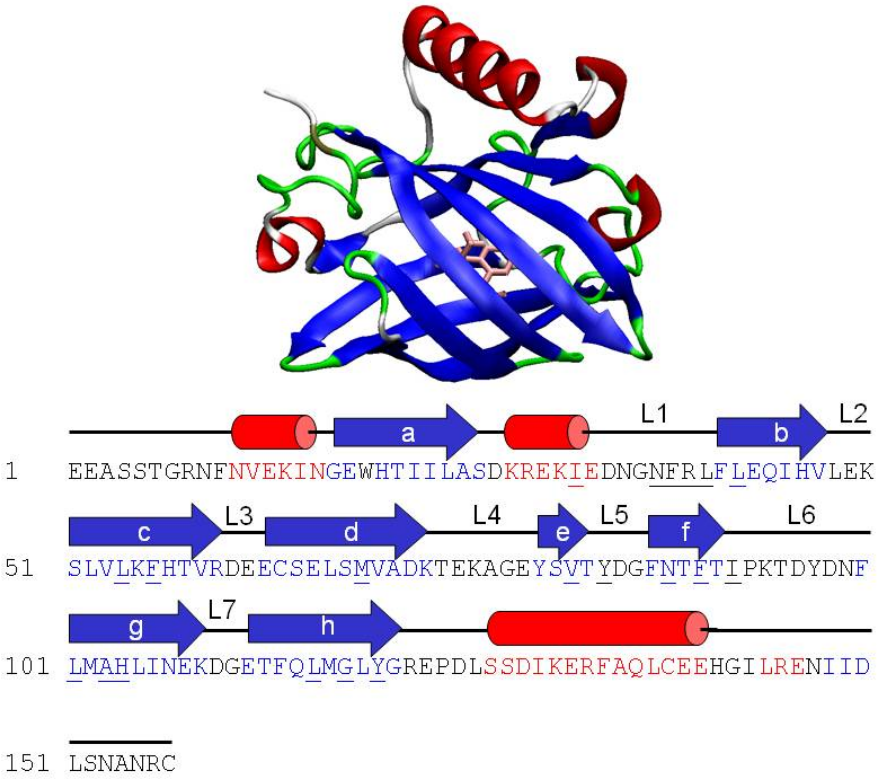

Figure S1. Sequence and structure of MUP. Underlined residues contact the bound IBM ligand (pink)

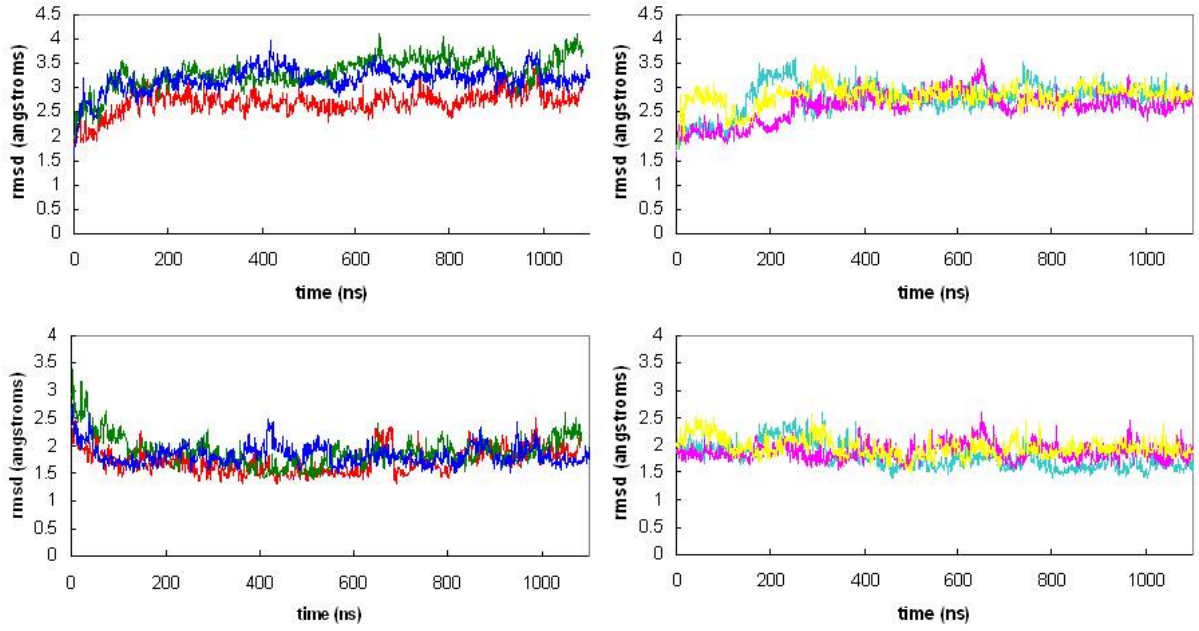

Figure S2. RMSD profiles for the first 1.1 microseconds of each simulation. Apo1: red; apo2: blue; apo3: green; ibm1: cyan; ibms2: magenta; ibm3: yellow. Top: RMSD from the respective crystals structures, below: RMSDs from the time-averaged structures

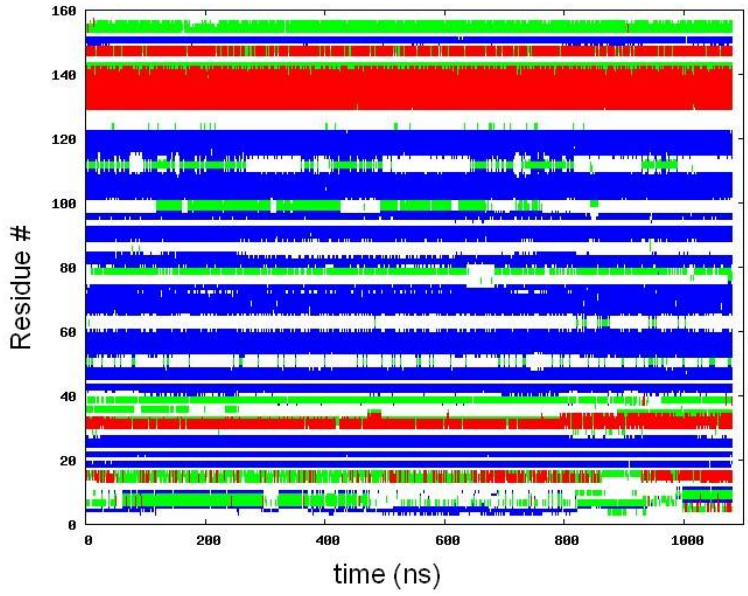

Figure S3. Secondary structure conservation over the first 1.1 microseconds of simulation apo1. Colour coding of secondary structure is as in Figure 1 in the main paper: red: alpha helix; blue: beta strand; green: turn.

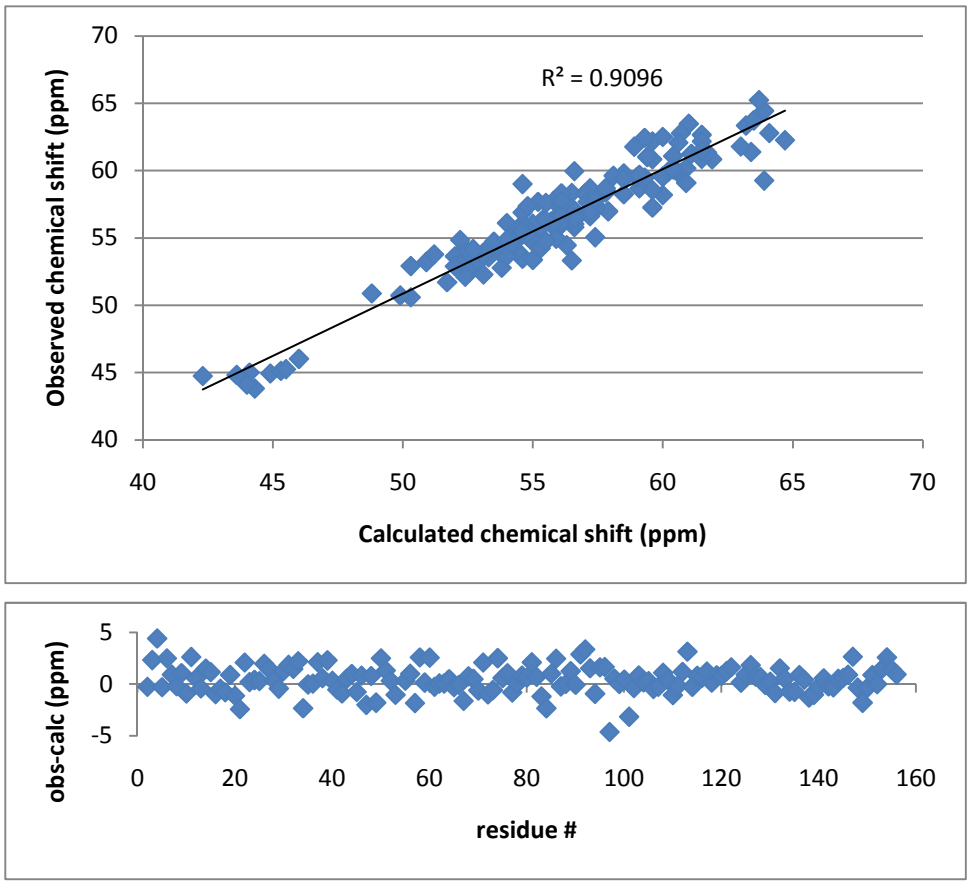

Figure S4. Top: Correlation between predicted Calpha chemical shifts (average calculated from 20 snapshots taken from simulation apo1 using CamShift) and values reported in BMRB entry 1470. Bottom: Errors between calculated and observed values.

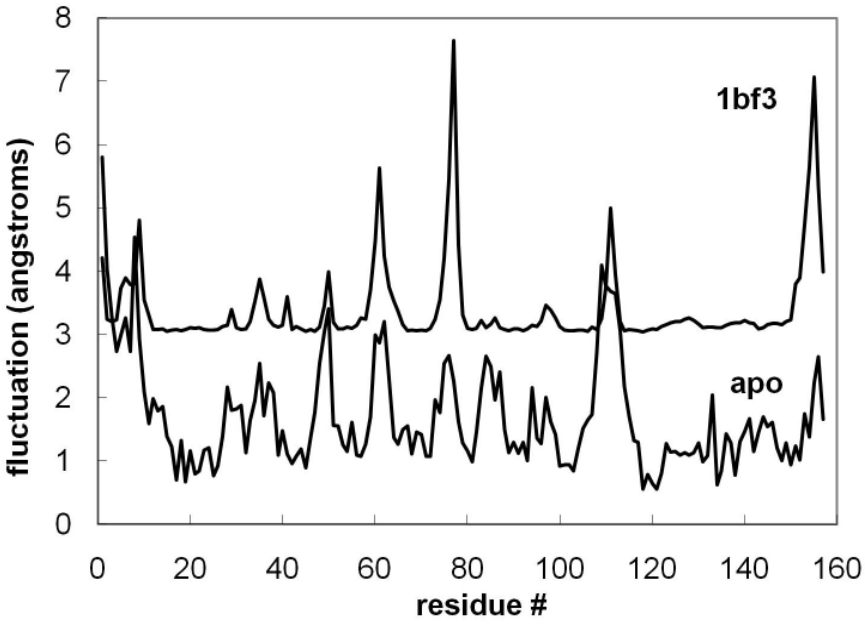

Figure S5. Comparison of the average of the RMS fluctuation profiles for the three apo simulations with that calculated from the ten structures for apo MUP in PDB entry 1BF3. The NMR data has been displaced vertically by 3 angstroms.

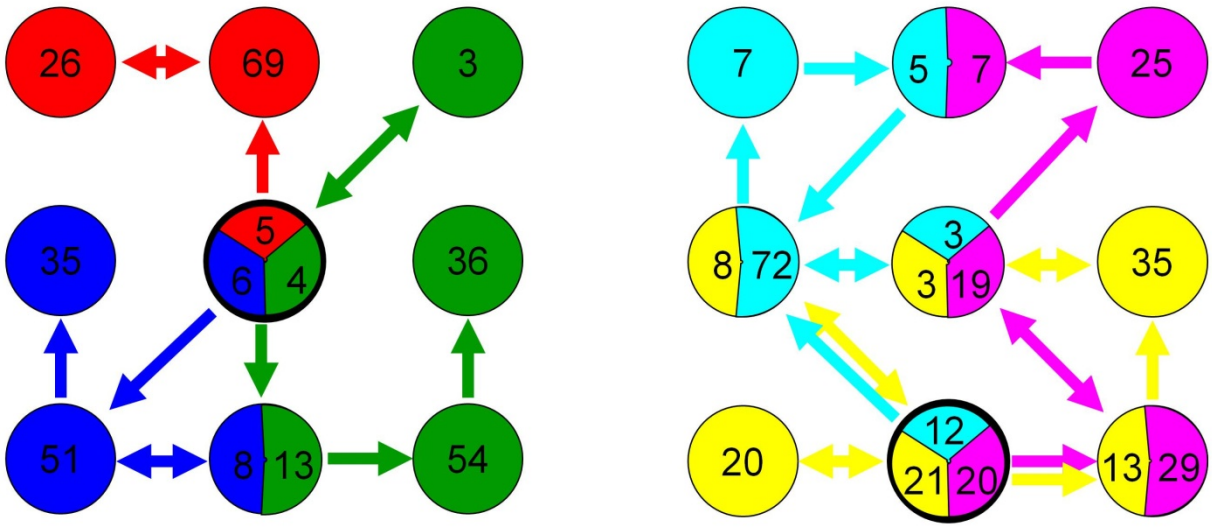

Figure S6. Cluster analysis of apo- (left) and ibm- (right) simulations. The clusters containing the crystal structures are outlined in black. Numbers in circles give the percentage occupancy of each cluster, arrows indicate cluster transitions. Colour coding is: apo1: red; apo2: blue; apo3: green; ibm1: cyan; ibms2: magenta; ibm3: yellow.

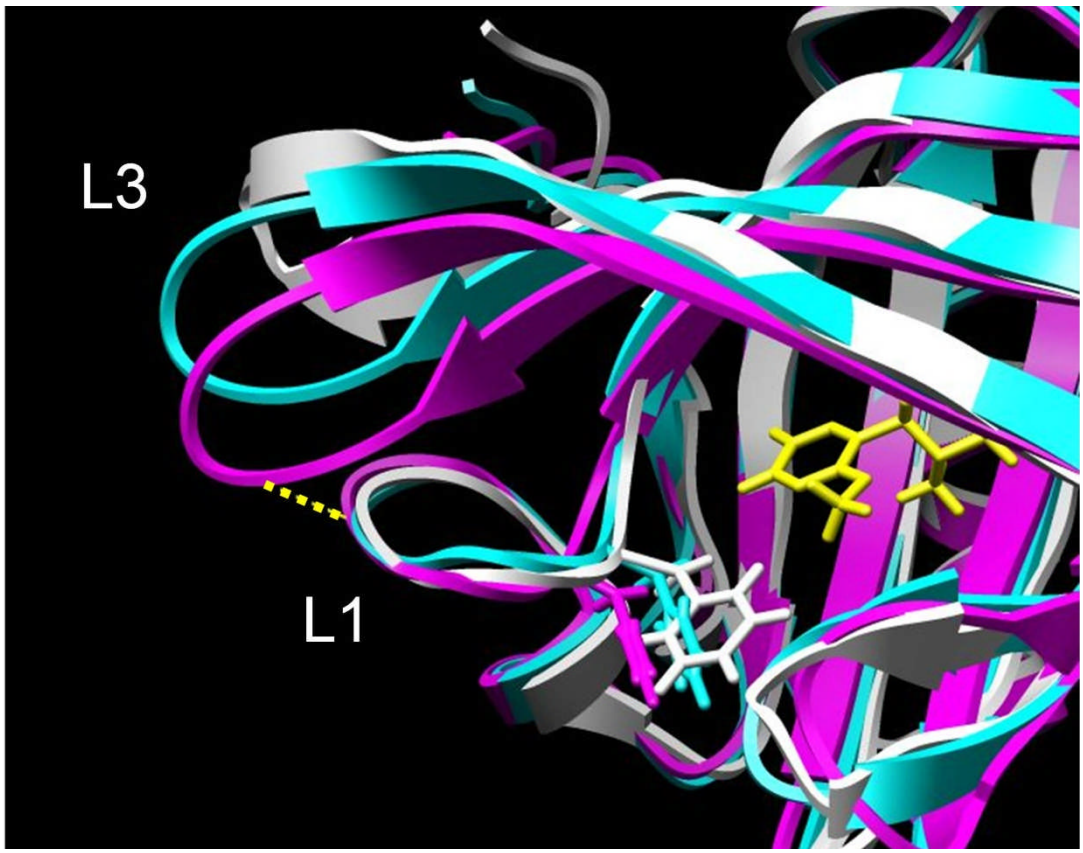

Figure S7. Comparison of the L3 regions of the apo- crystal structure (white), time-averaged structure from the three apo- simulations (magenta), and time-averaged structure from the three ibm- simulations (cyan). Phe38 and the ibm ligand (yellow) are also shown.

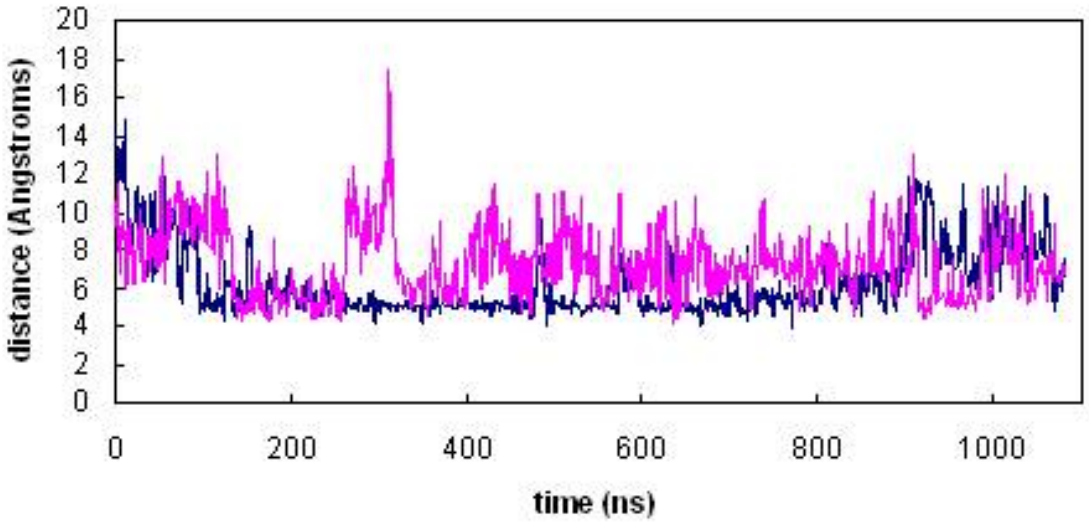

Figure S8. L3 motion in simulations apo1 (blue) and ibm1 (magenta) measured in terms of the distance between the C-alpha atoms of residues N35 and D61.

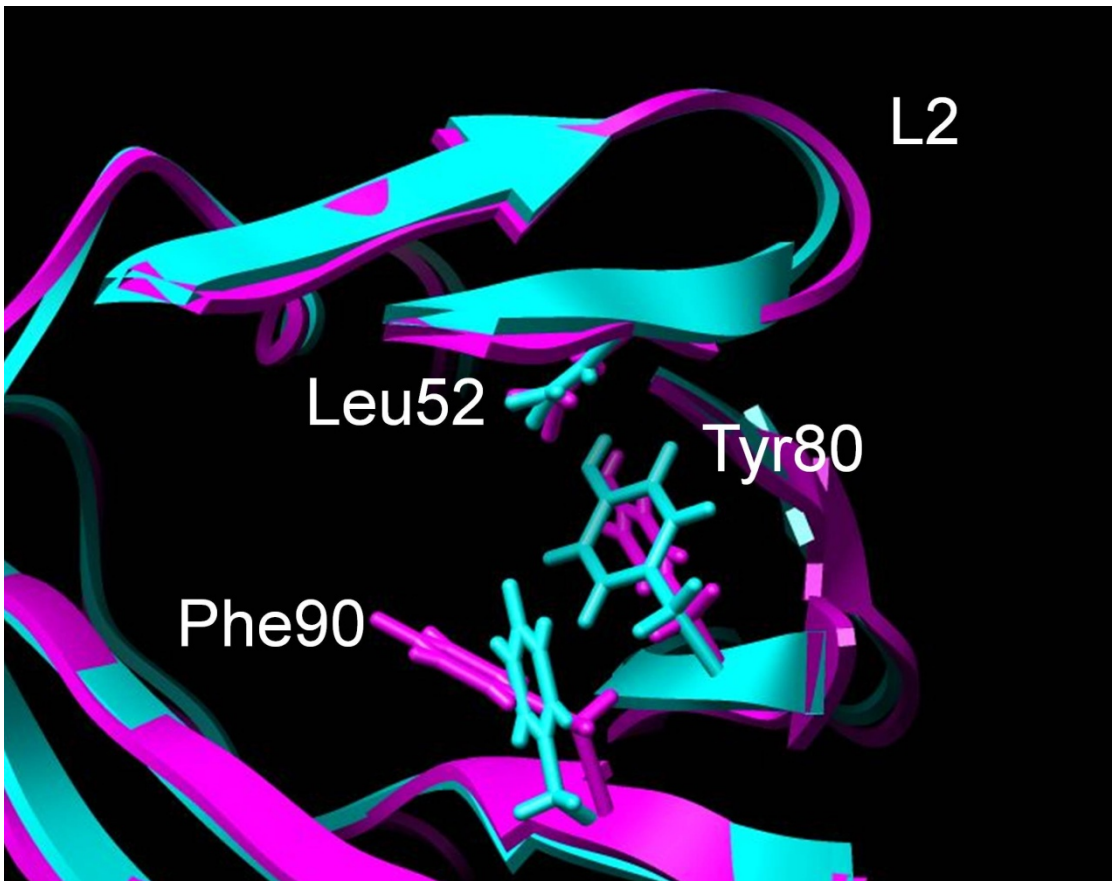

Figure S9. Comparison of the L2 region in the time-averaged structures from the apo- (magenta) and ibm- (cyan) simulations. Ligand binding shifts Phe90, which then packs tighter against Tyr80, which in turn rigidifies residues 48-50 via the interaction with Leu52.

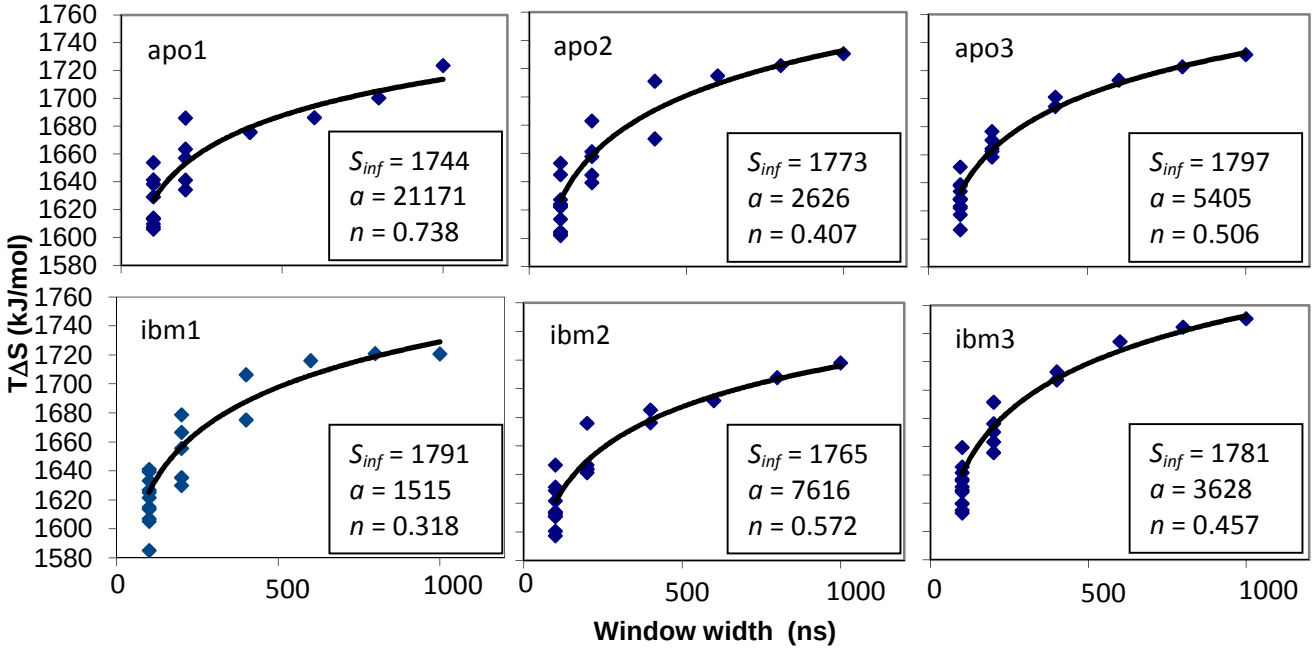

Figure S10. Schlitter entropy convergence. Values of S_{inf}, a and n from curve fitting are also shown.

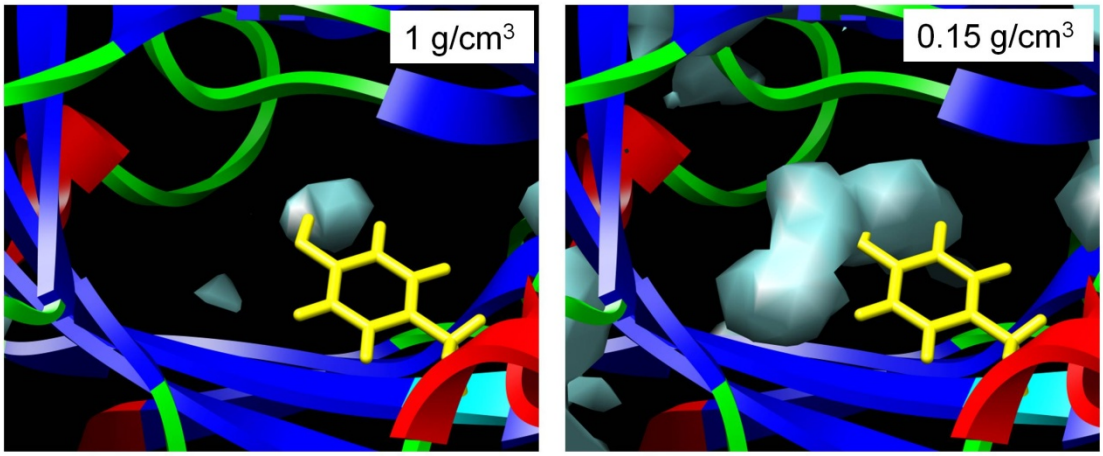

Figure S11. Water density in the ligand binding site of simulation apo1. The left panel shows the small volume with a 'normal' water density around the hydroxyl group of Tyr120, while the right panel is contoured at a low density to reveal the full extent of the binding pocket.

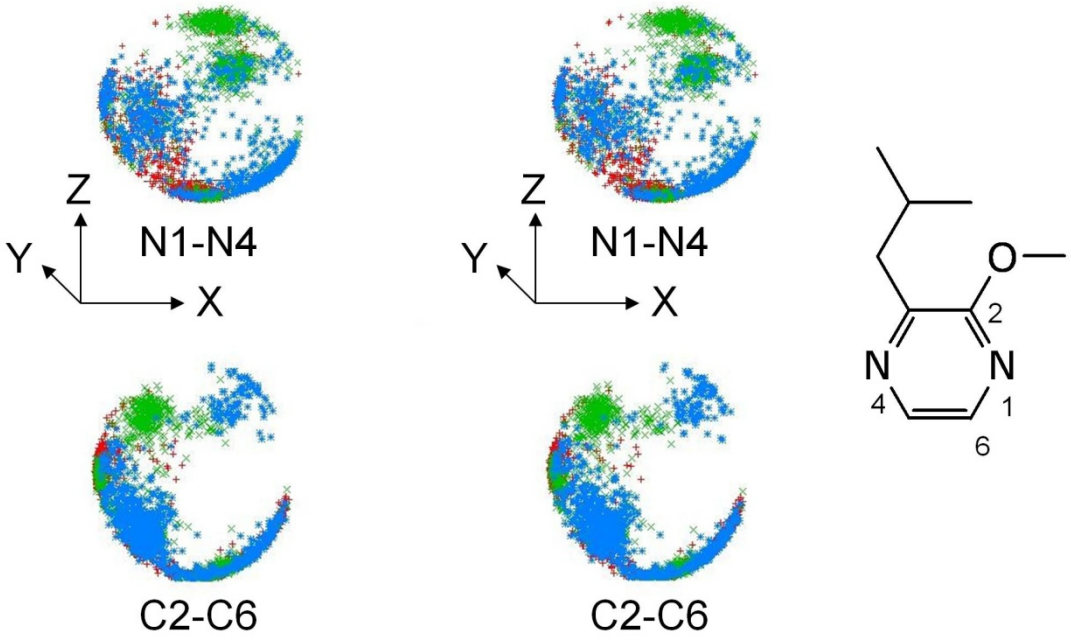

Figure S12. Stereoplots to illustrate ligand tumbling. Top: orientation of the N1-N4 vector, bottom: orientation of the C2-C6 vector. Red: simulation ibm1; green: ibm2; blue: ibm3.

“Prep” input file for the IBM ligand

```
    0    0    2

isobutyl methoxy pyrazine ligand for MUP. RESP charges. From Antechamber
molecule.res
IBM   XYZ  0
CORRECT     OMIT DU   BEG
  0.0000
   1  DUMM  DU    M    0  -1  -2     0.000      .0        .0      .00000
   2  DUMM  DU    M    1   0  -1     1.449      .0        .0      .00000
   3  DUMM  DU    M    2   1   0     1.522   111.1        .0      .00000
   4  C9    c3    M    3   2   1     1.540   111.208   180.000    -0.447
   5  H91   hc    E    4   3   2     1.088    63.585    39.670     0.107
   6  H92   hc    E    4   3   2     1.086    97.726   145.793     0.107
   7  H93   hc    E    4   3   2     1.086    50.837  -107.553     0.107
   8  C8    c3    M    4   3   2     1.531   150.154   -46.861     0.423
   9  H8    hc    E    8   4   3     1.086   107.891  -107.949    -0.026
  10  C10   c3    3    8   4   3     1.531   110.673    10.570    -0.447
  11  H101  hc    E   10   8   4     1.087   110.538   -58.025     0.107
  12  H102  hc    E   10   8   4     1.084   111.994  -178.171     0.107
  13  H103  hc    E   10   8   4     1.087   110.978    61.563     0.107
  14  C7    c3    M    8   4   3     1.543   109.736   134.685    -0.432
  15  H71   hc    E   14   8   4     1.084   109.527   -67.126     0.124
  16  H72   hc    E   14   8   4     1.086   108.771    50.390     0.124
  17  C2    ca    M   14   8   4     1.508   114.647   172.289     0.254
  18  N1    nb    M   17  14   8     1.302   119.517   102.903    -0.446
  19  C6    ca    M   18  17  14     1.338   118.949  -179.861     0.059
  20  H6    h4    E   19  18  17     1.073   117.131   179.923     0.099
  21  C5    ca    M   19  18  17     1.364   121.003    -0.214    -0.010
  22  H5    h4    E   21  19  18     1.074   121.789  -179.914     0.128
  23  N4    nb    M   21  19  18     1.338   121.411    -0.164    -0.493
  24  C3    ca    M   23  21  19     1.294   117.088     0.325     0.671
  25  O11   os    M   24  23  21     1.333   120.518  -179.705    -0.418
  26  C12   c3    M   25  24  23     1.411   118.306     0.216    -0.095
  27  H121  h1    E   26  25  24     1.081   110.928   -60.780     0.097
  28  H122  h1    E   26  25  24     1.080   105.593   179.830     0.097
  29  H123  h1    E   26  25  24     1.081   110.848    60.557     0.097


LOOP
   C2   C3

IMPROPER
   C7   C3   C2   N1
   C5   H6   C6   N1
   C6   H5   C5   N4
   C2   N4   C3   O11

DONE
STOP
```

(end of file)

Forcefield parameters for the IBM ligand

```
IBM parameters
MASS

BOND

ANGLE
c3-ca-nb   62.732     122.770   Calculated with empirical approach
nb-ca-os   71.521     121.235   Calculated with empirical approach
ca-os-c3   62.700     118.150   same as c2-os-c3

DIHE

IMPROPER
c3-ca-ca-nb         1.1          180.0         2.0          Using default value
ca-h4-ca-nb         1.1          180.0         2.0          Using default value
ca-nb-ca-os         1.1          180.0         2.0          Using default value

NONBON
```

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