Hydrogen bonding PMF parameters

Numbers of points in (x,y,z) (62, 62, 33)
Offsets in angstrom of the PMF box in (x,y,z) (-3.1, -3.1, -0.5)

E(x,y,z) PMF force constant multiplier, optimal range 0.20 - 0.25
E(θ''|r) PMF force constant multiplier, optimal range 0.03 - 0.10

ID of the (x,y,z), (θ''|θ), and (φ'|φ') potential classes
1  310 helix (i/i+3), right-handed
2  310 helix (i/i+3), left-handed
3  alpha helix (i/i+4), N-terminal or center
4  alpha helix (i/i+4), C-terminal or isolated turn
5  beta sheet (|i-j|>4), antiparallel, center or short cycle
6  beta sheet (|i-j|>4), antiparallel, long cycle
7  beta sheet (|i-j|>4), parallel, center
8  beta sheet (|i-j|>4), parallel, edge
9  long range (|i-j|>4), isolated

ID of the (θ''|r) potential classes
1  all i/i+3  CO/HN
2  all i/i+4  CO/HN
3  all |i-j|>4  CO/HN
An example of a CNS input file for the simulated annealing schedule.

```plaintext
define( md.seed=823641; )

parameter
  @parallhdg.pro
end

structure
  @1bld.mtf
end

parameter
  nbonds
    repel=0.8
    rconst = 4.0
    rexp=2 irexp=2
    nbxmod = 3
    wmin=0.01
    cutnb=5.0
    tolerance=0.5
end

noe
  nres=10000
  set message=off echo=off end
  class noe  @1bld_noes.inp
  set message=on echo=on end
  ceiling=100
  averaging  * r-6
  potential  * softsquare
  soexponent * 1
  sqexponent * 2
  sqconstant * 1.
  sqexponent * 2
  scale      * 5.
  rswitch    * 3.0
  asymptote * 1.0
end

restraints dihedral
  reset
  nassign=500
  set message=off echo=off end
  @1bld_dihe.inp
  set message=on echo=on end
  scale=200.
end
```

S2
set seed=&md.seed end
do (fbeta=10) (all)
do (mass= 25) (all)

flags exclude * include bond angle impr vdw noe cdih end
igroup
   interaction (all) (all) weights * 1. end
end

evaluate ( $nfile = 20 )
evaluate ( $ifile = 1 )

evaluate ( $imax = $nfile+1 )
while ( $ifile < $imax ) loop main

evaluate ($filename="1bld_"+encode($ifile)+".pdb")
coor initialize end
coor @@$filename

evaluate ( $t_ini = 1000.0)
evaluate ( $t_fin = 1.0)
evaluate ( $tsteps = 100)
evaluate ( $nsteps = 1000)
evaluate ( $dt = ($t_ini-$t_fin)/$tsteps )

evaluate ($t_curr = $t_ini)
evaluate ($nsim = 1)
evaluate ($nmax = $tsteps+2)
while ( $nsim < $nmax ) loop cool
    do (vx=maxwell($t_curr)) ( all )
do (vy=maxwell($t_curr)) ( all )
do (vz=maxwell($t_curr)) ( all )
dynamics cartesian
cmremove=false
tcground=false
tcoupling=true
timestep=0.001
nstep=$nsteps
nprint=1000
 temperature=$t_curr
end

evaluate ($t_curr = $t_curr-$dt)
evaluate ($nsim = $nsim +1)
end loop cool
evaluate ($filename="1bld_hb_"+encode($ifile)+".pdb")
set print=$filename end
print threshold=0.3 noe
print threshold=5.0 cdih
remarks overall, bonds, angles, impr, noe, dihe
remarks energies:$ener,$bond,$angl,$impr,$noe,$cdih
write coordinates output =$filename end
evaluate ($ifile = $ifile +1)

end loop main

stop
The B1 domain of protein G:
validation of the HB PMF with respect to dipolar couplings.

The experimental data deposition for 3GB1 differs from the earlier 1GB1/2GB1 in two important aspects. First, it includes an extensive set of residual dipolar coupling data (HN-N, HN-C and N-C couplings in two alignment media), which enable NMR-based structure validation. Second, some of the distance restraints deposited with the 3GB1 were reassigned with respect to 1GB1/2GB1, by itself resulting in a decrease of the backbone r.m.s.d. relative to the 1.9 Å resolution X-ray structure, 1PGB. These restraints were among those that were identified as being consistently violated by our refinement of 2GB1, as reported in tables 5 and 6.

The structure refinement protocol and the semi-empirical force field were the same as previously discussed, except for the terms used for fitting the deposited scalar and residual dipolar couplings. J-couplings were fitted with a quadratic flat-bottom potential and constant force constant of 1.0 kcal/Hz. Residual dipolar couplings were fitted with a flat-bottom quadratic potential and a force constant ramped exponentially from 0.01 to 1.0 kcal/Hz at the previously established constant values of the alignment tensor magnitude and rhombicity (-9.9 Hz/0.23 for the bicelles and -6.5 Hz/0.62 for the phage). All validation quality factors (Q-factors) for the dipolar couplings were calculated by SVD fits on the individual structures. Two sets of calculations were performed, one with the dipolar couplings included as input restraints, the other without. Each set was performed both with and without the HB PMF. The results of the trials are summarized in Supporting Information Table 1.
### Supporting Information Table 1. Structural refinement statistics for 3GB1.

<table>
<thead>
<tr>
<th>no dipolar couplings</th>
<th>with dipolar couplings</th>
<th>1PGB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB PMF</td>
<td>HB PMF</td>
<td></td>
</tr>
<tr>
<td>backbone r.m.s.d. to 1PGB</td>
<td>0.82±0.11</td>
<td>0.66±0.09</td>
</tr>
<tr>
<td>% in the most favored Ramachandran area</td>
<td>86.3±3.1</td>
<td>90.2±2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>dipolar coupling Q factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HN-N, bicelle</td>
<td>0.424±0.057</td>
</tr>
<tr>
<td>HN-N, phage</td>
<td>0.476±0.087</td>
</tr>
<tr>
<td>N-C, bicelle</td>
<td>0.508±0.051</td>
</tr>
<tr>
<td>N-C, phage</td>
<td>0.502±0.053</td>
</tr>
<tr>
<td>HN-C, bicelle</td>
<td>0.575±0.052</td>
</tr>
<tr>
<td>HN-C, phage</td>
<td>0.652±0.042</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HB PMF average values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>E(r,θ,φ)</td>
<td>-3.01±0.24</td>
</tr>
<tr>
<td>E(θ''</td>
<td>r)</td>
</tr>
</tbody>
</table>

---
