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.

```
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
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
```

```
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($t_curr)) ( all )
    dynamics  cartesian
      cmremove=false
      vscaling=false
      tcoupling=true
      timestep=0.001
      nstep=$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[1] differs from the earlier 1GB1/2GB1 in two important aspects. First, it includes an extensive set of residual dipolar coupling data (H^N-N, H^N-C and N-C couplings in two alignment media), which enable NMR-based structure validation.[2] 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^2. 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^2 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).[1] All validation quality factors (Q-factors)[2a] 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.

		no dipolar couplings		with dipolar couplings		1PGB
		no HB PMF	with HB PMF	no HB PMF	with HB PMF	
backbone r.m.s.d. to 1PGB		0.82±0.11	0.66±0.09	0.71±0.04	0.63±0.03	0.00
% in the most favored Ramachandran area		86.3±3.1	90.2±2.1	94.8±1.4	94.2±1.4	90.0
dipolar coupling Q factors	HN-N, bicelle	0.424±0.057	0.289±0.030	0.062±0.001	0.056±0.002	0.244
	HN-N, phage	0.476±0.087	0.390±0.058	0.066±0.003	0.057±0.002	0.281
	N-C, bicelle	0.508±0.051	0.423±0.030	0.227±0.002	0.212±0.003	0.377
	N-C, phage	0.502±0.053	0.445±0.039	0.238±0.013	0.225±0.008	0.371
	HN-C, bicelle	0.575±0.052	0.502±0.023	0.244±0.004	0.230±0.005	0.443
	HN-C, phage	0.652±0.042	0.593±0.043	0.299±0.004	0.283±0.006	0.493
HB PMF average values	$E(r,\theta,\phi)$	-3.01±0.24	-4.99±0.07	-3.57±0.07	-4.28±0.10	-4.71
	$E(\theta''\|r)$	0.94±0.17	0.29±0.06	0.46±0.08	0.24±0.02	0.36

(1) (25) Kuszewski, J.; Gronenborn, A. M.; Clore, G. M. *J. Am. Chem. Soc.* **1999**, *121,* 2337-2338.

(2) (a) Cornilescu, G.; Marquardt, J.L.; Ottiger, M; Bax, A. *J. Biomol. NMR* **1998**, *120*, 6836-6837.; (b) Clore, G. M., Garrett, D. S. *J. Am. Chem. Soc.* 1999, *121,* 9008-9012.