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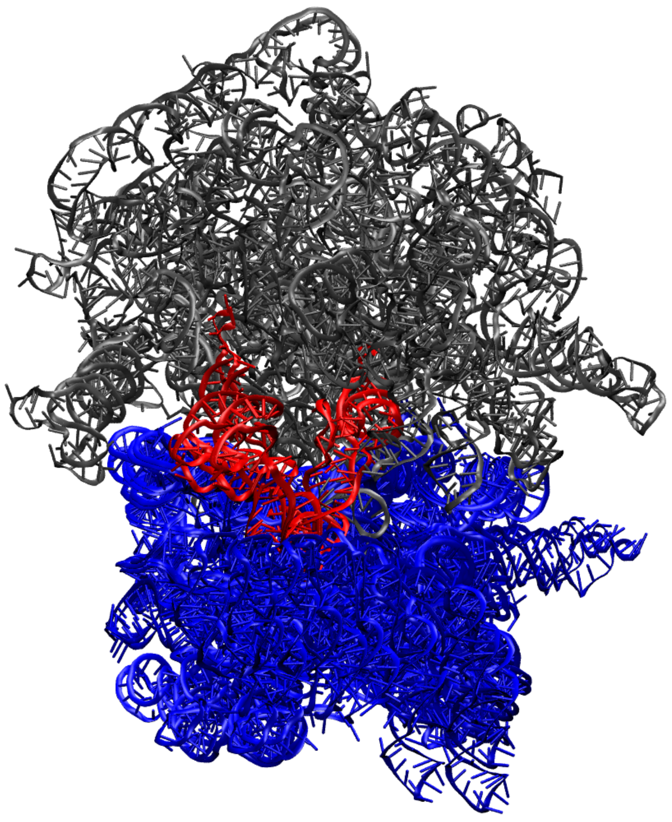
MMB 2.8.2



Reference guide

May 14, 2012

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1 What's new?

In release 2.8 I made more improvements to MMB. Most significantly, you can now have a biopolymer which has gaps (e.g. unresolved residues) in the input structure file. MMB will then match to the available coordinates and guess at a loop conformation which connects those fragments. There is no guarantee that the loop will be clash free (but we have tools to then resolve clashes). You can choose between `matchGapped` (which uses default, chemically reasonable bond lengths and angles) and `matchGappedNoHeal` (for certain special cases -- this will typically have a physically unreasonable bond geometry between the inserted region and the second fragment of known structure, requiring annealing). You can also use `matchFast`, which is very economical and works perfectly with MMB's own double-precision output structure files.
MMB can now read the chain ID's sequence, residue numbers, and even insertion codes from an input PDB file, for RNA and protein (not DNA or other species). We tolerate gaps in numbering. You can even use the '+' operator to specify *relative* residue numbers. The shorthand `FirstResidue` and `LastResidue` are now supported. You will note changes in syntax for some commands, but as before the error messages should coach you in modifying your old command files to work with release 2.8. In addition, we did some more internal restructuring, in the way `constrainToGround`'s, `atomSpring`'s are handled, though this is invisible to the user.

For any published work which uses MMB, please cite one or more of the following:

Turning limited experimental information intio 3D models of RNA, by Samuel C Flores and Russ B Altman, *RNA* 16(9):1769-78 (2010).
Predicting RNA structure by multiple template homology modeling, by Samuel C. Flores, Yaqi Wan, Rick Russell, and Russ B. Altman (2010) *Proceedings of the Pacific Symposium on Biocomputing.*

2 Biopolymers and monoAtoms

In this Appendix, we describe how to instantiate biopolymers (RNA, protein), as well as single atoms such as counterions. Note that the number of biopolymers and series of single atoms is limited by the number of characters available as chain identifiers.

2.1 Biopolymer sequences and first residue numbers

MMB can instantiate RNA chains using the following syntax:

```
RNA <chain ID> <first residue #>  <sequence in single letter code>
```

Similarly, you can instantiate DNA chains like this:

```
DNA <chain ID> <first residue #>  <sequence in single letter code>
```

You can instantiate a protein chain as:

```
protein <chain ID> <first residue #> <sequence in single letter
code>
```

The protein chains use a the 20 canonical amino acid alphabet for specifying the sequence.

There is one more way to instantiate sequences, which works for protein and RNA. You can issue the command:

```
loadSequencesFromPdb
```

And MMB will go to your input structure file (last.??.pdb) and look for RNA and protein chains. It will extract the chain ID's, residue numbers, insertion codes, and residue types from there. It will also match the internal coordinates to the Cartesian coordinates it finds there, as usual. You will then be able to issue commands that involve residues in those chains, as before.

In addition to removing the need for you to specify these chains manually, the also has the advantage of handling insertion codes and gaps in the numbering. You will be able to append an insertion code to the right of the residue number in *any* command, e.g. `constrainToGround A 32B` (where B is an insertion code). Further, it is now also possible to use the '+' operator to increment or decrement a residue ID by some number of residues. For instance,

```
constrainToGround A 32B+2
constrainToGround A 32B+-3
```

will constrain residues two residues to the C-terminus and three residues to the N-terminus of 32B.

The residue numbers and insertion codes do need to be increasing from the top to the bottom of the input structure file, though. Before using this command, you should clean up the input structure file, removing anything that is not RNA or protein – including DNA, water, ions, or other molecules.

2.2 monoAtoms

The `monoAtoms` command specifies single atoms (e.g. monatomic ions) The syntax follows:

```
monoAtoms <chain ID> <first residue #> <# of atoms> <name of atom>
```

Currently only the following atom names are supported:

```
Mg+2, Cl-, Na+, K+, Li+, Ca+2, Cs+, Rb+
```

The single atoms created with this command support the `atomSpring`, `atomTether`, `springToGround`, `constrainToGround`, and `constraint` commands, just like the biopolymers. They do not support the `mobilizer` command.

3 Forces

In this Appendix, we describe options for using the `baseInteraction, aromatic` two-residue forces, the `atomSpring, atomTether,` and `springToGround forces`, and the `contact` steric forces. Note that since forces are additive, there is no hard limit on how many forces can exist in the system or even acting on a single residue, base, or atom.

3.1 baseInteraction

The syntax for this command is:

```
baseInteraction <chain identifier for first residue>
           <residue number for first residue>
           <interacting edge for first residue>
           <chain identifier for second residue>
           <residue number for second residue>
           <interacting edge for second residue>
           <glycosidic bond orientation>
```

The following combinations of first base pairing edge, second base pairing edge, and glycosidic bond orientation are permitted:

```
WatsonCrick WatsonCrick Cis
WatsonCrick WatsonCrick Trans

WatsonCrick Hoogsteen Cis
WatsonCrick Hoogsteen Trans

WatsonCrick SugarEdge Cis
WatsonCrick SugarEdge Trans

Hoogsteen Hoogsteen Cis
Hoogsteen Hoogsteen Trans

Hoogsteen SugarEdge Cis
Hoogsteen SugarEdge Trans

SugarEdge SugarEdge Cis
SugarEdge SugarEdge Trans

WatsonCrick Bifurcated Cis
Stacking3 Stacking5 Cis
Stacking5 Stacking5 Trans
```

```
Stacking3 Stacking3 Trans
HelicalStackingA3 HelicalStackingA5 Cis
Superimpose Superimpose Cis
```

You might notice that some of these are actually not in the Leontis and Westhof classification. These are explained below:

- Stacking* simply specifies a stacking interaction between consecutive residues on a chain. The numbers indicate which face is interacting on each base. For example:
  `baseInteraction A 120 Stacking3 A 121 Stacking5 Cis`

  Means that the face of base 120 which would ordinarily point towards the 3' end of the strand in a helix, will be stacked on the face of base 121 which would ordinarily point to the 5' end of the helix.

- HelicalStacking* works the same as Stacking, but adds the offset appropriate for consecutive bases in a helix. HelicalStackingA3/HelicalStackingA5 is automatically applied to all consecutive bases in helices, unless you specify `setHelicalStacking FALSE`. MMB assumes an A-form helix exists whenever it finds three consecutively numbered RNA residues on a single strand Watson-Crick base paired with three consecutively numbered residues on the same or another single RNA strand. If you want to generate a helix where this is not the case, you should manually apply HelicalStackingA3 / HelicalStackingA5 interactions.

3.2 nucleicAcidDuplex

This command generates WatsonCrick/WatsonCrick/Cis interactions between two specified segments on the same or different RNA chains. It is a shortcut for manually specifying each such interaction for every pair of canonically interacting residues in the duplex. The syntax is:

```
nucleicAcidDuplex     <chain identifier A>
                      <first residue on A>
                      <last residue on A>
                      <chain identifier B>
                      <first residue on B>
                      <last residue on B>
```

Recalling that the duplex is antiparallel, we require that:

```
(first residue on A) < (last residue on A)
```
and
```
(first residue on B) > (last residue on B)
```

For example:

```
nucleicAcidDuplex A 1 3 A 10 8
```

Makes the segments between residues 1 and 3 (inclusive) and between 10 and 8 (inclusive) into two halves of a duplex, by applying a base pairing interaction between 1 and 10, 2 and 9, and 3 and 8.

3.3 atomSpring

The `atomSpring` command creates a linear spring connecting two atoms. Two optional parameters (square braces []) specify the dead length and spring force constant.

```
 atomSpring   <first chain ID>
              <first residue number>
              <first atom name>
              <second chain ID>
              <second residue number>
              <second atom name>
              [<dead length>
              [<spring constant>]]
```

3.4 atomTether

The `atomTether` command, as the name implies, applies no force if the distance between atoms is less than a certain `<dead length>`, and applies an attractive force with Hookean `<spring constant>` when the distance exceeds the former. Default values for the last two parameters are 0.0 and 3.0, respectively, as they are for `atomSpring`. Make `<spring constant>` large for a strict “dog leash” or small for a permissive restraint.

```
 atomTether   <first chain ID>
              <first residue number>
              <first atom name>
              <second chain ID>
```

```
          <second residue number>
          <second atom name>
          [<dead length>
          [<spring constant>]]
```

3.5 springToGround

The `springToGround` command creates a linear spring connecting a specified atom and a specified location in Ground. Two optional parameters (square braces []) specify the dead length and spring force constant.

```
springToGround   <atom chain ID>
                 <atom residue number>
                 <atom name>
                 <X location in Ground>
                 <Y location in Ground>
                 <Z location in Ground>
                 [<dead length>]
```

3.6 threading

The `threading` command applies `atomSpring`'s between pairs of atoms with the same name, on corresponding residues. The result is that the atoms of a given stretch of residues in a given chain 1 are aligned to the like-named atoms of a corresponding stretch in a second chain 2. For release 2.6.2 and higher, this command works with any biopolymer; its predecessor only worked for protein. The optional parameter (square braces []) specifies the spring force constant.

```
threading   <chain 1 ID>
                    <first residue number of chain 1>
                    <last  residue number of chain 1>
                    <chain 2 ID>
                    <first residue number of chain 2>
                    <last  residue number of chain 2>
                    [<spring constant (default = 3.0)>]
```

If you are trying to align just the backbone of a protein, you can use the `proteinBackboneThreading` command, which has the same syntax as above, but only applies springs between corresponding N, CA, and C atoms.

3.7 contact

You can also apply space-filling Contact spheres to a range of residues using the `contact` command. (The idea is similar to that of the parameters `addSelectedAtoms` and `addAllHeavyAtomSterics`)

```
contact    <contact type>
           <chain identifier>
           <residue number for first residue>
           <residue number for last residue>
```

The first residue should be lower numbered than the second, and both residues should be on the same chain.

There are two kinds of permitted values of `contact type`. In the fixed type, the atom identities are hard-coded and can't be modified by the user, but the contact sphere radii and stiffness (both of which are the same for all atoms regardless of atom name) correspond to the `excludedVolumeRadius` and `excludedVolumeStiffness` parameters which are set in the MMB input file (e.g. commands.dat). These include:

`AllAtomSterics` : Puts one sphere on each atom of the chain, except for the end caps on proteins (when used).
`AllHeavyAtomSterics` : Puts one sphere on each atom of the chain EXCEPT hydroges, and again except for the end caps on proteins.
`RNABackboneSterics` : Puts one sphere on each of the following atoms: P, O5*, C5*, C4*, C3*, and O3*. An error will result from attempting to apply this to proteins, as anytime when you attempt to put sterics on an atom which doesn't exist on a given residue.

The second type of sterics are user configurable, in the parameter file (e.g. parameters.csv). Here the user can choose on which atoms to put the spheres, with a maximum of four atoms. The radii and stiffness can be controlled separately for each atom name. A different choice of zero to four atom names can be chosen for each residue type (4 residue types for RNA, 20 for protein). The user can add as many steric schemes to the parameter file as he/she wishes; as supplied the `parameters.csv` file has two: `SelectedAtoms` and `ProteinBackboneSterics`. For the first one, the parameters look like:

RECORD	A		SelectedAtoms	SelectedAtoms	X	P	C4*	N9
RECORD	C		SelectedAtoms	SelectedAtoms	X	P	C4*	N1
RECORD	G		SelectedAtoms	SelectedAtoms	X	P	C4*	N9
RECORD	U		SelectedAtoms	SelectedAtoms	X	P	C4*	N1

The second column is the residue type, and columns 7,8, and 9 are the atom names. Note that the glycosidic nitrogen is named differently for purines vs. pyrimidines. Subsequent columns give the sphere radii, stiffnesses, and information to identify these as `contact parameter` entries. Parameters become available for use immediately upon being entered in the parameter file, much as for MD force field parameter files.

3.8 Restraining to ground

Much as residues can be constrained to each other (see next chapter), any residue of any chain can also be restrained to ground, meaning that a force can be applied to pull all six translational-rotational degrees of freedom to an equilibrium position and orientation in Ground:

```
restrainToGround <chain ID> <residue number>
```

Keep in mind that unlike a constraint, a restraint acts as a spring and thus allows some displacement with respect to ground. Any displacement at the end of a stage is carried over to the next stage, potentially leading to a “creeping” effect. Two parameters which are relevant to this command are `restrainingForceConstant` and `restrainingTorqueConstant`. These set the translational and angular restitution force constants.

3.9 Density based force field

As explained in the tutorial, MMB’s density based force field is formulated following Klaus Schulten’s MDFF as follows:

$$\vec{f}_i = A \cdot m_i \cdot \vec{\nabla} D(x_i, y_i, z_i)$$

Where *i* is the atom index, m_i is the mass of atom *i*, $D(x_i, y_i, z_i)$ is the electronic density at the nuclear position of atom *i*, A is a user-adjusted scaling factor, and $\vec{\nabla}$ is the gradient operator. Accordingly, $\vec{f}_i$ is the density-derived force vector applied to atom *i*. This is computed for and applied to every atom *i* in the system.

To turn on the density based force field on or off, set:

```
densityMapActivate    <True | False>
```

By default, if `densityMapActivate` is `True`, all chains in the system will be subject to density based forces. If you only want a limited number of chains to be fitted, with the remaining chains not subjected to these forces, just specify each chain to be fitted with a command like this:

```
fitToDensity <chain ID>
```

... one for each chain, of course.

Your density map must be in XPLOR format. To specify the location of the density map, file, use:

```
densityFileName <density file name>
```

The scaling factor (A in the equation above) defaults to unity, but you can set it to any floating point number (including negative numbers) as follows:

```
 densityForceConstant  <scale factor>
```

3.10 Physics where you want it

"Physics where you want it," introduced in release 2.4, allows you to turn on the all-atoms force field only for certain regions of your system, referred to as the "physics zone."

To turn this feature on or off, use:

```
physicsWhereYouWantIt <True | False>
```

This parameter defaults to `False,` meaning the force field terms are applied to all atoms. Set to `True` to restrict to a user specified set.

To specify a range of residues to be added to the physics zone, use:

```
includeAllNonBondAtomsInResidues <chain ID> <first residue in range>
<last residue in range>
```

Sometimes it will be convenient to include all residues within a certain radius of a specified residue. For this you would use:

```
includeAllResiduesWithin <distance> <chain ID> <residue number>
```

The `distance` (in Å) is measured between key atoms, CA for protein and C4* for RNA and DNA.

Lastly, we have found that small chemical groups such as methyl or alcohol can spin out of control in the absence of viscous forces, leading to small time steps and excessive computational expense. To deal with this, you can scale the inertia of such small groups with:

```
smallGroupInertiaMultiplier <inertia scale factor>
```

Any nonnegative floating point number can be used here; we suggest 11.0.

4 Mobilizers and constraints

In this Appendix we describe `mobilizer` commands, which define or modify the internal coordinate topology of the molecule as well as `constraint` commands, which add constraint equations that reduce the degrees of freedom of the system.

It is important to keep in mind the crucial difference between these two in Internal Coordinate Mechanics. A `mobilizer` command can reduce or increase the number of bodies that exist in a system; in the former case you will always save computer time. On the other hand a `constraint` command adds constraint equations which must then be solved; while the net effect depends on masses and forces, computational cost typically increases. Mobilizers control bond mobilities, which here can be `Free`, `Torsion`, or `Rigid`.
`Free` means that the bond can change its length, angle, and dihedral.
`Torsion` means it can change only its dihedral angle.
`Rigid` means it has no degrees of freedom.

One must also avoid overconstraining the system. For example, if two rigid molecules are already `Weld`'ed (see below) to each other, do not put additional constraints on this pair of molecules, even if they are nominally applied to different residues. While this is easy to keep track of for two bodies, watch out for more insidious ways of overconstraining. For example, if A is `Weld`'ed to B, and B is `Weld`'ed to C, do not then `Weld` C to A.

4.1 mobilizer

The `mobilizer` keyword is used for specifying the bond mobilities for a stretch of residues. This command is overloaded. The first variant has the following syntax:

```
mobilizer  <bond mobility>
           <chain identifier>
           <first residue number>
           <last residue number>
```

The first residue should be lower numbered than the second, and both residues should be on the same chain. Bond mobility can be set to `Free`, `Torsion`, `Rigid`, or `Default`.

Don't forget you can use the keywords `FirstResidue` or `LastResidue`, or do arithmetic on the residue numbers using the "+" operator, as described earlier.

You can also simply say:

```
mobilizer  <bond mobility>
           <chain identifier>
```

... and this will set ALL residues in chain `<chain identifier>` to `<bond mobility>`.

Lastly, you can say:

```
mobilizer  <bond mobility>
```

... and this will set all residues in ALL chains to `<bond mobility>`.

4.2 applyMobilizersWithin

The `applyMobilizersWithin` command is used to specifying the bond mobilities for all biopolymer residues within a certain radius of a specified residue. It has the following syntax:

```
applyMobilizersWithin <bond mobility>
                      <radius>
                      <chain identifier>
                      <residue ID>
```

The `radius` is measured between representative atoms (Cα for protein, C4* for nucleic acids) and (like always) in Å. The acceptable values of `<bond mobility>` are as listed above.

4.3 mobilizeInterfaces

The `mobilizeInterfaces` command is used to specifying the bond mobilities for all biopolymer residues within a certain distance of all interfaces of a given biopolymer chain. The syntax is:

```
mobilizeInterfaces    <interface depth>
                      <bond mobility>
                      <chain identifier>
```

The `<interface depth>` is measured as the minimum distance between representative atoms (Cα for protein, C4* for nucleic acids) on different chains across an interface. `<chain identifier>` is the chain whose interfaces you are interested in.

As an example, if you have a trimer of chains A,B, and C, and issue :

```
mobilizeInterfaces 6.0 Torsion C
```

Then all residues at all interfaces between chain C and A, and between C and B (but not between A and B), to a depth of 6.0Å, will get bond mobility `Torsion`.

4.4 singleBondMobility

The `singleBondMobility` command is used for specifying the bond mobility for a single bond:

```
singleBondMobility    <chain identifier for first atom>
           <residue number for first atom>
           <atom name for first atom>
           <bond mobility>
           <chain identifier for second atom >
           <residue number for second atom >
           <atom name for second atom>
```

The two atoms should be covalently bonded to each other, of course.

4.5 Default mobilizers

It is important to understand what is the default setting for the mobilizers in your system.

The default bond mobility leaves most bonds set to `Torsion`, but there are also some `Rigid` bonds, depending on the residue type and atoms it connects. For instance, the bond mobilities for an RNA residue look like this:

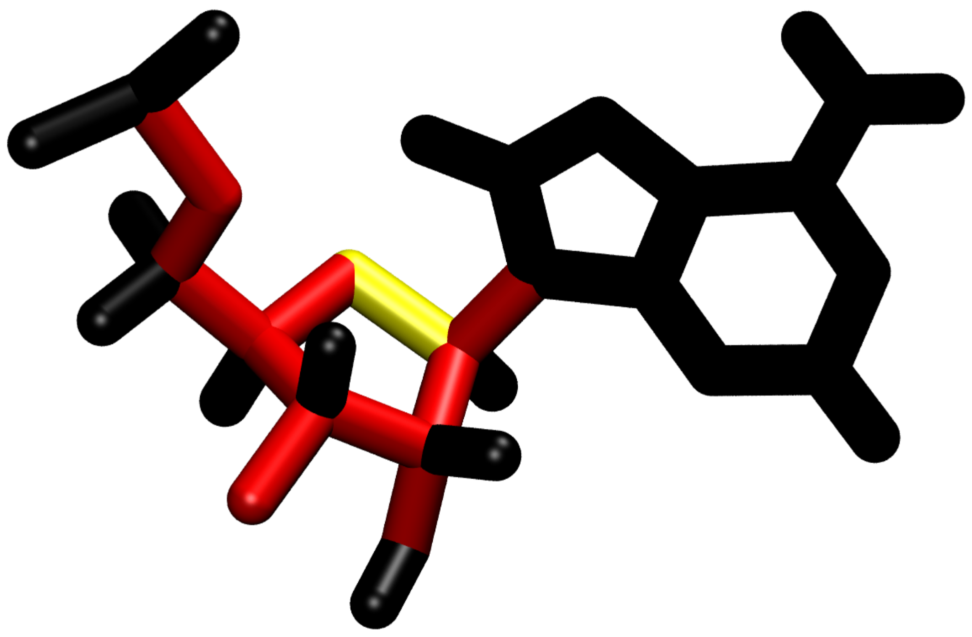

Figure 1 : Default bond mobilities for an RNA residue

Black: `Rigid`; **Red:** `Torsion`; **Yellow:** `Free`.

Similarly for a protein residue, most bonds are also `Torsion`, but the peptide bond is `Rigid`, as are some cyclic side chain groups.

4.6 Order of application of mobilizers

In order to get the desired result out of MMB, you should understand the order in which these commands are applied. They go like this:

1. `mobilizer`
2. `applyMobilizersWithin`
3. `mobilizeInterfaces`
4. `singleBondMobility`

A common mistake is to forget that before any commands are applied, all chains have a default bond mobility, as described above.

4.7 constraint

The `constraint` command is used for specifying constraints to weld two residues together:

```
constraint      <chain identifier for first residue>
                <residue number for first residue>
                Weld
                <chain identifier for second residue>
                <residue number for second residue>
```

The two welded residues can be on different chains; in fact either or both residues can be in RNA or protein chains. The weld is applied on C3* atoms of RNA residues and on C atom s of protein residues. There is no preference for residue number ordering.

4.8 Constraining to ground

Just as residues can be constrained to each other, any residue of any chain can also be constrained (rigidly attached) to ground:

```
constrainToGround <chain ID> <residue number>
```

See *Appendix: Parameters* for an explanation of the `constraintTolerance` parameter, relevant to this command.

5 Global parameters

This appendix, describes global parameters available to users. It does not cover *commands* such as `baseInteraction, aromatic, contact, mobilizer,` and `constraint.` The simplest difference between a *parameter* and a *command* is the following. A *command* can be issued an unbounded number of times, subject only to memory and computer time limitations. The major caveat is that in the case of `constraint` commands, one must not overconstrain the system. In contrast a *parameter* can only be set once (at least for a given stage); if a parameter is set multiple times for a given stage, only the last value of that parameter will be used. A listing of all user-configurable global parameters and their current values is printed at the beginning of every stage of an MMB run. Some additional parameters are available but rarely used or not recommended; contact the author with questions on these.

This chapter does not describe *staged* parameters. These are parameters for which not only the *value,* but also the *stage* at which they first take effect is specified, for example `temperature` and `dutyCycle`.

addAllAtomSterics	Bool	FALSE	Add steric contact spheres to all atoms. This is more expensive and more prone to kinetic trapping than addSelectedAtoms.
addAllHeavyAtomSterics	Bool	FALSE	Add steric contact spheres to all atoms EXCEPT hydrogens.
checkSatisfied	Bool	FALSE	At each reporting interval, list all the baseInteraction's and determine which were satisfied.
constrainRigidSegments	Bool	FALSE	When TRUE, adds a constrainToGround command for each rigid fragment in each chain in the system. Useful for equilibrating small regions in multiple points throughout a complex without affecting parts of the complex distant to those small regions.
constraintTolerance	float	0.05	This determines the tolerance of the Weld constraint. If Weld'ed pieces are moving relative to each other, reduce this number.
cutoffRadius	float	0.1	This is the range of the MMB potential. See our Multiple-template homology modeling paper.
densityFileName	String		Name of file for fitting based on electron density, in .xplor format. If you need to convert from some other format, we recommend using mapman (e.g. rave_osx for mac). Instructions are here:

			http://xray.bmc.uu.se/usf/mapman_man.html#S10
densityForceConstant	Float	1	Scale factor for the density based forces
densityMapActivate	bool	False	When True, turns on density based forces
firstStage	int	1	Stage at which simulation should begin.
globalAmberImproperTorsionScaleFactor	float	0	These eight parameters set scaling factors for terms in the Amber potential. Most default to 0 for economy.
globalBondBendScaleFactor	float	1.0	
globalBondStretchScaleFactor	float	1.0	
globalBondTorsionScaleFactor	float	0	
globalCoulombScaleFactor	float	0	
globalGbsaScaleFactor	float	0	
globalVdwScaleFactor	float	0	
initialSeparation	float	20.0	Sets the separation between chains at stage 1, or whenever readPreviousFrameFile = false.
integratorAccuracy	int	0.001	Integrator tolerance, applies for variable step size time integrators.
integratorStepSize	int	0.001	Step size in ps, for fixed step size integrators.
integratorType	string	Verlet	Choose between Verlet, RungeKuttaMerson
integratorUseFixedStepSize	Bool	FALSE	self explanatory
lastStage	int	1	Stage at which simulation will end
leontisWesthofInFileName	string	./parameters.csv	MMB parameter file
loadTinkerParameterFile	Bool	FALSE	If FALSE, uses hard-wired Tinker parameters. If 1, reads parameters from tinkerParameterFileName
numReportingIntervals *alias* maxReportingIntervals	int	100	Number of reporting intervals per stage.
nastGlobalBondTorsionScaleFactor	int	10	Scale factor for NAST torsional potential
randomizeInitialVelocities	Bool	FALSE	Adds a random velocity to each body at the beginning of the simulation stage. Note that if you are have any non-interacting bodies (e.g. free ions with charges turned off) you may wish to apply initial velocities, otherwise the Nose-Hoover thermostat will leave them in their zero kinetic energy state.
reportingInterval	float	0.2	Duration of reporting intervals, in ps.
rigidifyFormedHelices	int	FALSE	
scrubberPeriod	float	4	Duration of one cycle of potential rescaling (ON time + OFF time).
safeParameters	Bool	TRUE	When TRUE, checks for syntax errors as well as some potentially dangerous parameter values.
setForceAndStericScrubber	Bool	FALSE	If TRUE, when dutyCycle < 1.0, turns ALL forces (including baseInteraction's, sterics, Amber force field, springToGround's, etc.) off for (dutyCycle -1) of the time.
setHelicalStacking	Bool	TRUE	if TRUE, identifies three consecutive WatsonCrick/WatsonCrick/Cis base pairs as a helix and applies HelicalStackingA3/HelicalStackingA5/Cis baseInteraction's between the consecutive residues on each strand.
setTemperature	Bool	TRUE	Turns on thermostat.
thermostatType	string		Choices are NoseHoover and VelocityRescaling
tinkerParameterFileName	string		Name of the tinker-formatted parameter file. Only needed if the tinker force field is turned on.

baseInteractionForceMultiplier *alias* twoTransformForceMultiplier *alias* forceMultiplier	float	100	Scale factor applied to all baseInteraction and aromatic forces. 100 or 1000 is recommended to speed up modeling.
useFixedStepSize	Bool	FALSE	Specifies fixed-step-size time integration.

6 Macros

This appendix describes macros available to users. These macros set parameters on the user's behalf. These are provided in cases where the corresponding commands might be confusing to the user, or simply not under user control.

matchFast	See the chapter, "Matching to the input structure file." This sets matchExact TRUE, matchIdealized FALSE, and matchOptimize FALSE. It is the most economical set of matching parameters; see mentioned chapter for caveats.
matchGapped	See above mentioned chapter, This sets matchExact TRUE, matchIdealized TRUE, and matchOptimize TRUE. Use it when there are gaps in the input structure file. It will span the gaps using use default bond lengths and angles in the intervening fragment. Don't forget to adjust matchingMinimizerTolerance.
matchGappedNoHeal	Just like above, except will not attempt to optimize the fragment of unknown structure. The latter will be left at default bond lengths, angles, dihedrals, and overall location, and unnatural bonds will connect it to the fragments of known structure. Don't forget to adjust matchingMinimizerTolerance.

7 User defined variables, parameter arithmetic, and conditional blocks

In this Appendix, we describe how to define numerical variables, and various ways to specify sections of the input file which are to be read or ignored at certain stages.

7.1 Comment marker

The comment marker is `#`, e.g.:

```
# Don't read this, it's just a comment
```

7.2 User defined variables

User variables are defined with the following syntax:

```
@<variable-name> <float or integer value>
```

The variable `@<variable-name>` can then be used wherever a literal integer or float is expected. If a float is assigned to the variable, and the variable is later used where an integer is expected, MMB will return an error. The definition of the variable should precede its first use in the input file. For example:

```
#declare @myStage variable and set to 3
@myIntervals 3
# now use it where a number (in this case an integer) is expected:
numReportingIntervals @myIntervals
```

Don't use any punctuation or whitespace in `<variable-name>.`

Don't try to set `firstStage` or `lastStage` with a user variable.

7.3 Parameter arithmetic

User variables are pretty handy, and start to make the command file more like a programming language. In the same vein, MMB allows the '+' and '-' operators. This means that any integer or floating-point (double-precision) parameter value can be set using a combination of literals, user variables, and the above operators. There is no limit to the number of operators and operands. Here are a couple of examples:

```
@DUMMY 40
numReportingIntervals  @DUMMY+10-@DUMMY
@MYFLOAT 0.35
reportingInterval  4+@MYFLOAT-0
```

This is equivalent, of course, to:

```
numReportingIntervals  10
reportingInterval  4.35
```

Don't use any whitespace or additional punctuation (such as parentheses, commas, etc.) in an arithmetic expression. Note also that residue ID's are special (they're not integers), and their '+' operator follows different rules (see Chapter 2).

7.4 Conditional blocks

In many cases we will want to issue different commands and make different choices of parameter values at different stages of a job. For this purpose we can enclose a block of the input file in a conditional block, which is opened as follows:

`readFromStage <stage-number>` Read only if the current stage is equal to or GREATER than `<stage-number>.`

`readToStage` Read only if the current stage is equal to or LESS than `<stage-number>`.

`readAtStage` Read only if the current stage is EQUAL to `<stage-number>`.

`readExceptAtStage` Read only if the current stage is NOT EQUAL to `<stage-number>`.

The commands and parameters to be conditionally read follow, and the end of the block is indicated with a `readBlockEnd` statement, e.g.:

```
# start conditional block:
readAtStage 3
# read the following lines only at stage 3:
sequence C CCUAAGGCAAACGCUAUGG
firstResidueNumber C 146
baseInteraction A 2658 WatsonCrick A 2663 WatsonCrick Cis
contact C 146 SelectedAtoms C 164
# end conditional block:
readBlockEnd
# continue with the rest of the input file
```

8 Matching internal coordinates to the input structure file

8.1 Introduction

MMB has a highly flexible input structure file handler. The first part of our method is a PDB file writer which can write additional atomic coordinate records at double precision. The second part is a set of PDB file readers which take advantage of any PDB files which have been written using this extra precision (to save computer time), and which can even guess at the position of any atoms which are missing from the input structure file.

MMB can write PDB files which contain an additional line after each atom record, with higher-precision coordinates. The records look like this:

```
ATOM     73  CA  LEU g  11     187.037 166.544 295.833  1.00  0.00           C
REMARK-SIMTK-COORDS 187.03654248299949359 166.54394767275138634 295.83272525824793320
```

Notice that the `REMARK-SIMTK-COORDS` records follow the `ATOM` records, repeating the coordinates except with higher precision. The higher precision is not necessary to make the dynamics accurately – it is there to expedite the process of turning Cartesian to internal coordinates. This way bond lengths, angles, and dihedrals can be matched without significant accumulation of error. The `REMARK-SIMTK-COORDS` records will be ignored by other programs which read PDB files, since they start with `REMARK`. Our PDB file reader will look for one such record after each ATOM record, but will use the original, lower-precision coordinates if the former is not found or disagrees with the coordinates in the ATOM record. These records are always used in last.??.pdb (which is written at the end of each stage, see program flowchart in the Tutorial Guide) and frame.pdb (which always contains the latest frame, duplicating the latest frame written to trajectory.??.pdb). By default, the trajectory.??.pdb files do not have these extra-precision records, in the interest of saving disk space. If you want to override this default, set `writeDoublePrecisionTrajectories True`.

As mentioned the input file reader can use these extra records if they are available, but does not require them. If any atoms are missing, it can guess their positions using two different schemes. That is to say, where insufficient atom positions are known, bond lengths, angles and/or dihedral angles will be either left at default values or adjusted to connect the pieces of known structure. No attempt will be made to prevent steric clashes – this is something that you may need to fix later, e.g. using `contact` spheres.

There are three cases. First, is the case where either all atom positions are known, or the only missing atoms are in side chains or termini. Second is the case where there are is a fragment of unknown structure (FUS) between fragments of known structure (FKS's), and it is geometrically possible for the FUS to connect the two FKS's, while leaving all bond lengths and angles at default values. The third case is like the second, except that it is not possible for the FUS to span the gap without introducing bonds of unnatural length and/or angle.

8.2 Case 1

This is the "easy" case, in which the FKS for any given chain is continuous, that is there is no FUS flanked by two FKS's. Here we will match all bond lengths, angles, and dihedrals using the corresponding pairs, triples, and quadruples of atoms in the input structure file. It is the most economical method available. It works perfectly when double precision coordinates are available. However error can accumulate when only standard PDB precision is available, though this error is not always noticeable for smaller macromolecules. In the latter case one might want to use one of the other macros in following sections. For Case 1, just invoke the following macro:

```
matchFast
```

This method is so much more economical than the others that you will want to use it whenever possible. Thus if you have a multi-stage run, you should definitely use it after the initial stage, at which point you will have MMB-generated double-precision PDB files.

8.3 Case 2

This is a harder case, in which one or more given chains have discountinuous FKS's. That is, there is at least one FUS flanked by two FKS's. However it should be possible for the FUS to span the gap between the two FKS's without breaking the default bond lengths and angles in the FUS. Here the bond lengths, angles, and dihedrals in each FKS will be set using the corresponding pairs, triples, and quadruples of atoms in the input structure file, as before. Following this, there will be a nonlinear optimization of all dihedral angles to match the available Cartesian atomic coordinates. This optimization may still be off by a little bit, but the FUS should now be connecting the two flanking FKS's. Then there will be a seond round of matching the bond lengths, angles, and dihedrals in the FKS's followed by a simple minimization which can be almost perfect (error in FKS's as low as 0.01Å) – but for this you must set `matchingMinimizerTolerance`. A value of 0.15 or so works well. You can experiment with a higher value to save compute time, at the cost of some accuracy. Since there are two rounds of optimization, this is the most expensive method of the three. To do this, invoke:

```
matchGapped
```

8.4 Case 3

This is just like Case 1, except that it is NOT possible for the FUS to span the gap between the two FKS's without breaking its default bond lengths and angles. For instance, the distance between the two FKS's may be longer than the fully extended length of the FUS. This is an unnatural situation, but there may be reasons of convenience for it to arise. The fitting procedure is exactly the same as before, except that we will skip the nonlinear optimization of dihedral angles. As a result, the FUS will have default bond lengths, angles and dihedrals, and further will be translationally in a position that is not related to that of the FKS. The FKS coordinates can be matched almost perfectly – here again make sure you adjust `matchingMinimizerTolerance`. The connection between the FUS and each of the FKS's will typically be a bond of unnatural length and angle. It will be the user's responsibility to equilibrate this bond (e.g. using the `singleBondMobility` command to flexibilize it). To do the fitting in this way, invoke:

```
matchGappedNoHeal
```

This method is more expensive than `matchFast`, as the name of the latter would suggest. For the ribosome you can expect `matchGappedNoHeal` to take about six hours, though for more pedestrian molecules (a couple of hundred residues long, say) it should take seconds or minutes. It is less expensive than `matchGapped`, though.

8.5 Economizing your time

As mentioned, `matchFast` is the fastest matching method, and it works best with our double-precision input structure file records. Also as mentioned, the last.??.pdb (generated at the end of every stage) and frame.pdb (generated at every reporting interval) files have double precision coordinate records. So if it's necessary to use `matchGappedNoHeal` or `matchGappedNoHeal`, and the molecule is a large one, the trick is to use it only once.

Another trick is to get rid of any gaps in numbering, and just connect the residues spanning the gap with a bond of unphysical geometry. The way to do is to simply *renumber* the residues in the input structure file. Let's say you have *inserted* residues 167A, 167B, 167C, followed by 168. These become 167, 168, 169, and 170, and all subsequent residues are also incremented accordingly. Similarly, let's say residues 212-214 are deleted. Residues 211 and 215 would become 211 and 212. For this you will use the program `renumber.pl`, included with MMB. The syntax is:

```
renumber.pl [old structure file name] [first residue number] > [new structure file name]
```

The structure file should just have one chain.