On the use of intra-molecular distance and angle constraints to lengthen the time step in molecular and stochastic dynamics simulations of proteins

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Abstract

Computer simulation of proteins in aqueous solution at the atomic level of resolution is still limited in time span and system size due to limited computing power available and thus employs a variety of time-saving techniques that trade some accuracy against computational effort. An example of such a time-saving technique is the application of constraints to particular degrees of freedom when integrating Newton’s or Langevin’s equations of motion in molecular dynamics (MD) or stochastic dynamics (SD) simulations, respectively. The application of bond-length constraints is standard practice in protein simulations and allows for a lengthening of the time step by a factor of three. Applying recently proposed algorithms to constrain bond angles or dihedral angles, it is investigated, using the protein trypsin inhibitor as test molecule, whether bond angles and dihedral angles involving hydrogen atoms or even stiff proper (torsional) dihedral angles as well as improper ones (maintaining particular tetrahedral or planar geometries) may be constrained without generating too many artificial side effects. Constraining the relative positions of the hydrogen atoms in the protein allows for a lengthening of the time step by a factor of two. Additionally constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein does not allow for an increase of the MD or SD time step.

KEYWORDS

classical equations of motion, constraints, molecular dynamics, stochastic dynamics, trypsin inhibitor

1 | INTRODUCTION

The length of the time step $\Delta t$ in a molecular dynamics simulation is limited by the highest frequency ($\nu_{\text{max}}$) motions occurring in the system,

$$\Delta t < 1/\nu_{\text{max}}.$$  \hspace{1cm} (1)

$\nu_{\text{max}}$ can be decreased by freezing high-frequency internal vibrations, such as bond-length or possibly bond-angle or particular torsional-angle vibrations. This then allows for a longer time step $\Delta t$. However, although such internal vibrations are often not of primary interest, the application of constrained dynamics only makes sense physically and computationally when

1. the frequencies of the frozen (constrained) degrees of freedom are (considerably) higher than those of the remaining ones, thereby allowing for a (substantial) increase of $\Delta t$,

2. the frozen degrees of freedom are only weakly coupled to the remaining ones, that is, when the molecular motion is not significantly affected by the application of the constraints,
3. so-called metric-tensor effects play a minor role.
4. the algorithm by which the constraints are imposed on the molecular system does not require excessive computational effort.

Bond-length degrees of freedom in proteins largely satisfy these conditions. Their vibrational frequencies are higher than those of the other degrees of freedom, their oscillations are largely decoupled from the other motions in a molecule, metric-tensor effects are insignificant, and algorithms to impose distance constraints do not require excessive computational effort. A factor of three in computer time can typically be saved by the application of bond-length constraints.

Bond-angle constraints in molecules containing torsional-angle degrees of freedom do not satisfy the above-mentioned four conditions as well as bond-length constraints. Their vibrational frequencies are lower, their motions are less decoupled from other motions in a molecule, and metric-tensor effects can be significant. While small solvent molecules without torsional-angle degrees of freedom are commonly simulated as rigid molecules, in proteins, the effect of bond-angle constraints applied to all bond angles present in the molecule is significant: Flexibility and entropy are halved, and the number of torsional-angle transitions is reduced. Yet, bond angles involving hydrogen atoms might be constrained without too many artificial side effects. In addition, torsional-angle degrees of freedom, proper (torsional) ones as well as improper ones (maintaining particular tetrahedral or planar geometries), may also be constrained in order to remove their motions from the molecular system.

In bio-molecular systems a hierarchy of motional frequencies originating in different types of interatomic interactions can be distinguished. In order of decreasing frequency or increasing smoothness of the corresponding atom–atom interaction term in the biomolecular force field we have (table 2 of Reference 13): (i) Bond-stretching vibrations with an approximate oscillation or relaxation time \( \tau < 10 \) fs for bonds involving a hydrogen atom and \( \tau < 20 \) fs for bonds involving only carbon or heavier atoms. (ii) Bond-angle bending vibrations with \( \tau < 20 \) fs for bond angles involving hydrogen atoms and \( \tau < 40 \) fs for bond angles involving only carbon or heavier atoms. (iii) Improper dihedral angle vibrations due to force field terms used to impose planarity on ring structures with conjugated double bonds with \( \tau < 30 \) fs. (iv) Torsional-angle vibrations around double bonds (e.g., peptide bonds) with \( \tau < 30 \) fs. (v) Water or solvent librational vibrations with \( \tau < 30 \) fs. (vi) Torsional-angle vibrations around single bonds with \( \tau < 40 \) fs for torsional angles involving a hydrogen atom and \( \tau < 80 \) fs for torsional angles involving only carbon or heavier atoms. (vii) Motions dominated by (not softened) van der Waals contacts and short-range (e.g., hydrogen bonding) Coulomb interactions with \( \tau < 150 \) fs. (viii) Motions dominated by long-range Coulomb (ionic, dielectric) interactions with relaxation time \( \tau < 2000 \) fs. It is this hierarchy that is exploited to enhance the efficiency of a simulation through the use of longer time steps \( \Delta t \).

Several methods are available to apply distance constraints during a MD simulation based on equations of motion in Cartesian coordinates, of which the SHAKE method is the oldest, simplest and a very robust technique. In SHAKE there is a limit to the maximal displacement, induced by the unconstrained forces, allowed for the atoms involved in each individual constraint. This local convergence criterion means that SHAKE will fail to converge when the forces acting on the specific atoms in a given constraint become very large. Thus, a SHAKE failure can be used to detect an error in the simulation, specifically the presence and location of unphysically large forces. Bond angles can be constrained in a similar manner using the procedure SHAKEBAC, and the dihedral angles accordingly with the procedure SHAKEAD.

An alternative to the use of equations of motion in Cartesian coordinates and imposing constraints through Lagrange multipliers is to employ internal coordinates, bond lengths, bond angles, and torsional angles. The classical equations of motion have been formulated by Lagrange in a most general form

\[
\frac{d}{dt} \left( \frac{\partial L(q, \dot{q})}{\partial \dot{q}_i} \right) = \frac{\partial L(q, \dot{q})}{\partial q_i}, \quad i = 1, \ldots, N_{df} \tag{2}
\]

where \( q_i \) denote the generalized coordinates, \( \dot{q}_i \) their time derivatives and the Lagrangean \( L(q, \dot{q}) \) is the kinetic energy \( K(\dot{q}) \) minus the potential energy \( V(q) \) of the system which contains \( N_{df} \) degrees of freedom. When using Cartesian coordinates \( q \equiv x \), one has \( K(x) = (1/2)mx^2 \) and Equation (2) reduces to Newton’s equations of motion (for \( N_{df} \) degrees of freedom).

\[
\frac{d^2 x_i}{dt^2} = -\frac{\partial V(x_1, x_2, \ldots, x_{N_{df}})}{\partial x_i}, \quad i = 1, 2, \ldots, N_{df} \tag{3}
\]

For branched polymers, such as proteins, the choice of internal coordinates, bond lengths, bond angles, and torsional angles seems to be natural. However, the equations of classical dynamics (2) expressed in the \( N_{df} \) internal, generalized coordinates \( q_i = \theta_i \) is

\[
\sum_{i=1}^{N_{df}} \frac{d^2 \theta_i}{dt^2} = -\frac{\partial V(\theta_1, \theta_2, \ldots, \theta_{N_{df}})}{\partial \theta_i} - \sum_{j=1}^{N_{df}} b_j \left( \frac{d \theta_j}{dt} \right)^2 - \sum_{k=1}^{N_{df}} c_{ij} \left( \frac{d \theta_i}{dt} \right) \left( \frac{d \theta_j}{dt} \right), \quad i = 1, 2, \ldots, N_{df} \tag{4}
\]

can be considerably more complex than when expressed in Cartesian coordinates (Equation 3). They contain two additional summations over the number of degrees of freedom and two additional quadratic (i.e., nonlinear) terms in the generalized velocities, and the coefficients \( a_i, b_i, \) and \( c_{ij} \) depend on the atomic masses and the molecular topology of the protein considered. Therefore, these equations of motion will not be used here.

In the present article, it is investigated whether the application of bond-angle and dihedral-angle constraints, in combination with the commonly applied bond-length constraints, in a molecular dynamics (MD) or stochastic dynamics (SD) simulation of a protein may allow for a lengthening of the discrete time step \( \Delta t \) used to numerically integrate the equations of motion, thereby keeping the distortive effects of constraining particular degrees of freedom upon the motion of the
nonconstrained degrees of freedom small. In view of the above-mentioned hierarchy of motions in proteins, three sets of constraints were used (see Tables 1 and 2):

1. All bond lengths in the protein (constraint set BLC).
2. One bond angle and one improper dihedral angle per hydrogen atom that, in combination with the constrained bond to the hydrogen atom, rigidify the position of the hydrogen atom with respect to its covalently bound neighbor nonhydrogen atoms (constraint set BADAC[H]), see Table 1. Stiff torsional dihedral-angle degrees of freedom involving a hydrogen atom are also constrained. For hydrogen atoms in C–O–H groups, in Ser, Thr, and Tyr amino-acid residues, only the O–H bond length and the C–O–H bond angle are constrained, its C–C–O–H dihedral angle is not constrained.
3. All other (not involving hydrogen atoms) improper dihedral angles and the stiff peptide-plane torsional angle and other stiff torsional angles in side chains are constrained (constraint set ISDAC), see Table 2.

The small protein bovine pancreatic trypsin inhibitor (BPTI) was chosen as test case, as in earlier study of the effect of constraining bond-length and bond-angle degrees of freedom in MD simulation. Since the protein model of Reference 5 did not contain explicit hydrogen atoms, the effect of constraining their relative positions was not investigated. The effect of constraining the geometry of the peptide plane and other stiff torsional-angle degrees of freedom was also not investigated.

The protein is simulated in three different ways:

1. In vacuo using MD simulation (MD_vac), in order to test the degree of conservation of total energy, linear and rotational momentum of the protein as function of the type of constraint (set BLC, set BLC + BADAC[H], or set BLC + BADAC[H] + ISDAC) applied and the size of the MD time step $\Delta t$. The vacuum boundary condition was chosen, instead of the commonly used periodic boundary condition, in order to be able to use a very large (100 nm), de facto infinite, nonbonded interaction cut-off distance. This eliminates the nonbonded interaction cut-off error, because all atom pairs are used in the force calculation. The geometric error induced by the application of constraints is governed by $t_{\text{ol}}$, for the bond lengths, by $t_{\text{ol}}_{\text{BAC}}$ for the bond angles, and by $t_{\text{ol}}_{\text{DAC}}$ for the dihedral or torsional angles. Values of $t_{\text{ol}}_{\text{DC}} = 10^{-5}$ and $10^{-4}$, and $t_{\text{ol}}_{\text{BAC}} = t_{\text{ol}}_{\text{DAC}} = 0.001^\circ$ and 0.01$^\circ$ were used when solving the constraint equations. In order to monitor energy conservation, the system was not coupled to heat or pressure baths and the motions of the center of mass and around the center of mass were not removed during the simulation.
2. Solvated in water using MD simulation (MD_aq), that is, in a rectangular periodic box with minimum image periodic boundary conditions, with many (6295) explicit rigid water molecules, the standard way of protein simulation, in order to test the behavior of dynamical protein properties as function of the type of constraint (set BLC, set BLC + BADAC[H], or set BLC + BADAC[H] + ISDAC) applied and the size of the MD time step $\Delta t$. Again, values of $t_{\text{ol}}_{\text{DC}} = 10^{-4}$ and $10^{-5}$ were used for the bond lengths and $t_{\text{ol}}_{\text{BAC}} = t_{\text{ol}}_{\text{DAC}} = 0.01^\circ$ and 0.001$^\circ$ for the bond angles and dihedral angles when solving the constraint equations. The protein and water were separately coupled to a heat bath and the system was coupled to a pressure bath. The motion of the center of mass of the system was removed during the simulation.
3. In vacuo using SD simulation (SD_vac), that is, replacing explicit water molecules by frictional and stochastic forces on the protein atoms, which roughly mimic the solvation effect in order to test the behavior of various protein properties as function of the type of constraint (set BLC, set BLC + BADAC[H], or set BLC + BADAC[H] + ISDAC) applied and the size of the SD time step $\Delta t$. This type of simulation may be used for very large protein complexes, such as the nucleosome, which would require the presence of very many water molecules in the system to fill a periodic box containing the complex.

## METHODS

### 2.1 Potential energy function or force field

When solvated in water (MD_aq), the protein was modeled using the GROMOS bio-molecular force field 54A7,54B7. The rigid simple point charge (SPC) model was used to describe the 6295 water molecules in the rectangular periodic box. When simulating the protein in vacuo (MD_vac, SD_vac), the GROMOS bio-molecular force field 54B7 was used. The A-version of a GROMOS force field is the basic force field designed for molecules in solution or in crystalline form. The B-version is derived from the A-version in order to be used for simulating molecules in vacuo, where the dielectric screening effect of the environment is neglected. The atomic charges and van der Waals parameters are changed such that atom charge groups with a nonzero total charge are neutralized while maintaining the hydrogen-bonding capacity of the individual atoms.

When applying bond-length constraints, that is, constraint set BLC, the corresponding bond-stretching terms of the force field are not evaluated.

When applying the bond-angle and dihedral-angle constraint set BADAC[H], the bond-angle bending terms of the force field are not evaluated for all bond angles involving a hydrogen atom, and the improper dihedral-angle terms and the stiff torsional dihedral-angle terms of the force field are not evaluated for the dihedral angles involving a hydrogen atom. A stiff proper (torsional) dihedral angle has a force constant $k_{\text{D}} > 15$ kJ/mole in the cosine force-field term. We note that the force field contains more bond-angle terms involving hydrogen atoms than bond angles involving a hydrogen atom that are constrained. The force field also contains more covalent interaction terms (bond-stretching, bond-angle bending, dihedral-angle bending) force-field terms than the corresponding number of degrees of freedom. This implies that the number of constraints may be less than the number of force-field terms corresponding to the constraints that must not be evaluated when the constraints are applied. Each hydrogen atom should have not more than three constraints, for example,
| Amino-acid residue | Hydrogen atom | Constrained bond angle | Value constraint θ° ( ) | Constrained dihedral angle | Value constraint ξ° or φ° ( ) |
|--------------------|---------------|------------------------|--------------------------|-----------------------------|-------------------------------|
| N-terminus         | H1, H2        | H1-N-CA                | 109.5                    |                             |                               |
|                    | H2, H3        | H2-N-CA                | 109.5                    |                             |                               |
|                    | H3            | H3-N-CA                | 109.5                    |                             |                               |
| Backbone (not Pro)| H             | H-N-CA                 | 115.0                    | N-C(-1)-CA-H               | 0.0                           |
| Arg                | HE            | HE-NE-CD               | 116.0                    | NE-CD-CZ-HE                | 0.0                           |
|                    | HH11          | HH11-NH1-CZ            | 120.0                    | NE-CZ-NH1-HH11             | 0.0 or 180.0                 |
|                    | HH12          | HH12-NH1-CZ            | 120.0                    | NH1-HH11-HH12-CZ           | 0.0                           |
|                    | HH21          | HH21-NH2-CZ            | 120.0                    | NE-CZ-NH2-HH21             | 0.0 or 180.0                 |
|                    | HH22          | HH22-NH2-CZ            | 120.0                    | NH2-HH21-HH22-CZ           | 0.0                           |
| Asn                | HD21          | HD21-ND2-CG            | 120.0                    | ND2-HD21-HD22-CG           | 0.0                           |
|                    | HD22          | HD22-ND2-CG            | 120.0                    | CB-CD-HD21-HD22-CG         | 0.0 or 180.0                 |
| Gln                | HE21          | HE21-NE2-CD            | 120.0                    | NE2-HE21-HE22-CD           | 0.0                           |
|                    | HE22          | HE22-NE2-CD            | 120.0                    | CG-CD-NE2-HE21             | 0.0                           |
| Hisa               | HD1           | HD1-ND1-CG             | 126.0                    | ND1-CG-CE1-HD1             | 0.0                           |
|                    | HD2           | HD2-CD2-CG             | 126.0                    | CD2-CD-CE2-HD2             | 0.0                           |
|                    | HE1           | HE1-CE1-ND1            | 126.0                    | CE1-ND1-NE2-HE1            | 0.0                           |
| Hisb               | HD2           | HD2-CD2-CG             | 126.0                    | CD2-CD-CE2-HD2             | 0.0                           |
|                    | HE1           | HE1-CE1-ND1            | 126.0                    | CE1-ND1-NE2-HE1            | 0.0                           |
|                    | HE2           | HE2-NE2-CD2            | 126.0                    | NE2-CD2-CE1-HE2            | 0.0                           |
| Hish               | HD1           | HD1-ND1-CG             | 126.0                    | ND1-CG-CE1-HD1             | 0.0                           |
|                    | HD2           | HD2-CD2-CG             | 126.0                    | CD2-CD-CE2-HD2             | 0.0                           |
|                    | HE1           | HE1-CE1-ND1            | 126.0                    | CE1-ND1-NE2-HE1            | 0.0                           |
|                    | HE2           | HE2-NE2-CD2            | 126.0                    | NE2-CD2-CE1-HE2            | 0.0                           |
| Lysh               | HZ1           | HZ1-NZ-CE              | 109.5                    |                             |                               |
|                    | HZ1, HZ2      | HZ1-NZ-HZ2             | 109.5                    |                             |                               |
|                    | HZ2           | HZ2-NZ-CE              | 109.5                    |                             |                               |
|                    | HZ2, HZ3      | HZ2-NZ-HZ3             | 109.5                    |                             |                               |
|                    | HZ3           | HZ3-NZ-CE              | 109.5                    |                             |                               |
| Phe                | HD1           | HD1-CD1-CG             | 120.0                    | CD1-CD-CE1-HD1             | 0.0                           |
|                    | HD2           | HD2-CD2-CG             | 120.0                    | CD2-CD-CE2-HD2             | 0.0                           |
|                    | HE1           | HE1-CE1-CD1            | 120.0                    | CE1-CD1-CE2-HD2            | 0.0                           |
|                    | HE2           | HE2-CE2-CD2            | 120.0                    | CE2-CD2-CE3-HE2            | 0.0                           |
|                    | HZ            | HZ-CZ-CE1              | 120.0                    | CZ-CE1-CE2-HE2             | 0.0                           |
| Ser                | HG            | HG-OG-CE               | 109.5                    |                             |                               |
| Thr                | HG1           | HG1-OG1-CA             | 109.5                    |                             |                               |
| Trp                | HD1           | HD1-CD1-CG             | 126.0                    | CD1-CD-CE1-HD1             | 0.0                           |
|                    | HE1           | HE1-NE1CD1             | 126.0                    | NE1-CD1-CE2-HE1            | 0.0                           |
|                    | HE3           | HE3-CE3-CD2            | 120.0                    | CE3-CD2-CE3-HE3            | 0.0                           |
|                    | HZ2           | HZ2-CE2-CH2            | 120.0                    | CE2-CD2-CE3-HE2            | 0.0                           |
|                    | HZ3           | HZ3-CE3-CH2            | 120.0                    | CE3-CD2-CE3-HE3            | 0.0                           |
|                    | HH2           | HH2-CH2-CE2            | 120.0                    | CH2-CD2-CE3-HE2            | 0.0                           |
one bond-length, one bond-angle and one (improper) dihedral-angle constraint. For hydrogens in a –C–O–H group, there are only two constraints, the bond length and the bond angle. For hydrogens in a –C–N–H₂ group, there are only three bond-length and five bond-angle constraints.

When applying the improper and proper (torsional) angle constraint set ISDAC, the corresponding improper and stiff torsional dihedral-angle bending terms of the force field must not be evaluated. Again, the force field contains more dihedral bending force-field terms than the corresponding number of constrained degrees of freedom.

2.2 | Constraints

The implementation into simulation software of the constraints and the omission of particular force-field terms is not trivial. In the GROMOS simulation software⁴⁰ use of the bond-length constraint set BLC is simple. The constraints correspond exactly with the bond-stretching force-field terms, and thus the constraints are taken from these force-field terms. In the GROMOS simulation software, each type of force-field term can be switched on or off. So when applying the BLC constraints, the bond-stretching force-field terms are switched off.

The use of the bond-angle and dihedral-angle constraints set BADAC(H) is less straightforward. In that case, the constraints of Table 1 are used in the form of a constraint list for the functions SHAKEBAC²¹ and SHAKEDAC²³ that constrain bond angles and dihedral angles, respectively. In addition, all bond-angle terms and improper dihedral-angle terms involving hydrogen atoms are switched off. For the proper (torsional) dihedral angles not involving hydrogens, this cannot be done, because only the stiff ones should not be evaluated. So the stiff dihedral angles not involving hydrogen atoms should be removed either from the protein molecular topology or from the amino-acid residue building blocks²⁸ used to construct the protein molecular topology.

The bond lengths in the protein and water molecules and the bond-angle distance of the water molecules were kept rigid with a relative geometric precision of tol_DC = 10⁻⁴ or 10⁻⁵ using the SHAKE algorithm.⁷ The bond angles and the dihedral angles in the protein were kept rigid with a precision of tol_DC = 0.01° or 0.001° using the SHAKEBAC algorithm²¹ and tol_DAC = 0.01° or 0.001° using the SHAKEDAC algorithm²³, respectively. The procedure to determine the Lagrange multipliers that determine the constraints may fail (i) because reference vectors (distances) and vectors being modified (i.e., the atoms are moved much due to large forces) or (ii) because no convergence of the iterations over all constraints can be obtained within a set limit of 1000 iterations.

2.3 | Treatment of long-ranged interactions

When simulating the protein in vacuo using MD (MD_vac), the nonbonded interactions are calculated for all atom pairs in the protein. When simulating the protein solvated in a periodic box filled with water molecules (MD_aq), the nonbonded interactions were calculated using a triple-range method⁴⁵,⁴² with cut-off radii of 0.8/1.4 nm. Short-range (within 0.8 nm) van der Waals and electrostatic interactions were evaluated every time step based on a charge-group pair list.³⁹ Medium-range van der Waals and electrostatic interactions, between pairs at a distance larger than 0.8 nm and shorter than 1.4 nm, were evaluated every 10 fs, at which time point the pair list was updated, and kept constant between updates. Outside the larger cut-off radius (1.4 nm) a reaction-field approximation⁴³,⁴⁴ with a relative dielectric permittivity ɛ_RF = 78.5 and a ionic strength of zero (κ_RF = 0) was used. The relative dielectric permittivity in the cut-off sphere was ɛ_CS = 1.⁴⁵ Minimum-image periodic boundary conditions
Table 2 Improper and proper (torsional) dihedral angles not involving hydrogen atoms in the various amino-acid residues that define the set of constraints ISDAC

| Amino-acid residue | Constrained dihedral angle | Value constraint $\xi^0$ or $\phi^0$ (°) |
|--------------------|-----------------------------|----------------------------------------|
| Backbone           | C-CA-N(-1)-O                | 0.0                                    |
|                    | CA(−1)-C(−1)-N-CA           | 0.0 or 180.0                           |
| Backbone (not Gly) | CA-N-C-CB                   | 35.26                                  |
| Arg                | CZ-NH1-NH2-NE               | 0.0                                    |
|                    | CD-NE-CZ-NH1                | 0.0 or 180.0                           |
| Asn                | CG-OD1-ND2-CB               | 0.0                                    |
| Asp                | CG-OD1-OD2-CB               | 0.0                                    |
| Cys1               | CB(1)-SG(1)-SG(2)-CB(2)     | 90.0 or 270.0                          |
| Gin                | CD-OE1-NE2-CG               | 0.0                                    |
| Glu                | CD-OE1-OE2-CG               | 0.0                                    |
| Hisa/b/h           | NG1-CD2-CB                  | 0.0                                    |
|                    | ND1-CD2-NE2                 | 0.0                                    |
|                    | CD2-CD1-CE1                 | 0.0                                    |
| Ile                | CB-CG1-CG2-CA               | 35.26                                  |
| Leu                | CB-CD1-CD2-CG               | 35.26                                  |
| Phe                | CG-CD1-CD2-CB               | 0.0                                    |
|                    | CD1-CD2-CE2                 | 0.0                                    |
|                    | CD2-CD1-CE1                 | 0.0                                    |
|                    | CD1-CE1-CZ                  | 0.0                                    |
| Thr                | CB-OG1-CG2-CA               | 35.26                                  |
| Phe                | CG-CD1-CD2-CB               | 0.0                                    |
|                    | CD1-CD2-CE2                 | 0.0                                    |
|                    | CD2-CD1-CE1                 | 0.0                                    |
|                    | CD1-CE1-CZ                  | 0.0                                    |
| Tyr                | CG-CD1-CD2-CB               | 0.0                                    |
|                    | CD1-CD2-CE2                 | 0.0                                    |
|                    | CD2-CD1-CE1                 | 0.0                                    |
|                    | CZ-CE1-CE2-OH               | 0.0                                    |
| Val                | CA-CG1-CG2-CA               | 35.26                                  |
| C-terminus         | C-O1-O2-CA                  | 0.0                                    |

Note: Values for the constraints were taken from the GROMOS force field 54A7.36,38 An atom of the next residue in the polypeptide chain is indicated by (−1), of the preceding residue by (−2). Cys1: first Cys residue that forms a S-S bridge with residue Cys2. The atoms of Cys1 and Cys2 are indicated with (1) and (2), respectively. The values of the improper dihedral angle $\xi^0$ are 0° or 35.26°. The values for the stiff (force constant $\gamma=15$ kJ/mol in the cosine force field term) proper (torsional) dihedral angle $\phi^0$ are either 0° or 180° for planar groups, and 90° or 270° for the S-S bridge.

2.4 Simulation set-up and equilibration

The protein was simulated using the GROMOS bio-molecular simulation software.40,46 The leap-frog algorithm17,48 was used to integrate Newton’s or Langevin’s equations of motion.

The initial structure of the protein BPTI, including four internally hydrogen bonded water molecules, was taken from the Protein Data Bank (PDB),49 entry 1bpi.

The protein initial structure was first energy minimized in vacuo to release possible strain induced by small differences in bond lengths, bond angles, improper dihedral angles, and short nonbonded contacts between the force-field parameters and the X-ray structure. The resulting protein configuration was used as initial configuration for the MD and SD simulations in vacuo (MD_vac, SD_vac). In order to exclude the influence of the fast librational motions of water molecules, the four internal water molecules were not included in these simulations.

The MD simulation of the protein solvated in a periodic box with explicit water molecules required the addition of water molecules. For the simulation of the protein in aqueous solution (MD_aq), the protein was put into a rectangular box filled with water molecules, such that the minimum solute-wall distance was 1.0 nm, and water molecules closer than 0.23 nm from the solute were removed. This resulted in a box with 6295 water molecules for the initial protein structure. In order to relax unfavorable contacts between atoms of the solute and the solvent, a second energy minimization was performed for the protein in the periodic box with water while keeping the atoms of the solute harmonically position-restrained40 with a force constant of 25 000 kJ mol$^{-1}$ nm$^{-2}$. The resulting protein-water configuration was used as initial configuration for the MD simulation of the protein solvated in explicit water (MD_aq).

In order to avoid artificial deformations in the protein structure due to relatively high-energy atomic interactions still present in the system, the MD simulations were started at $T = 60$ K and then the temperature was slowly raised to $T = 300$ K. Initial atomic velocities were sampled from a Maxwell distribution at $T = 60$ K and the translation of and the rotation around the center of mass of the system were removed. The equilibration scheme consisted of five short 20 ps simulations at temperatures 60, 120, 180, 240, and 300 K, at constant volume. At 300 K, the equilibration was extended to 2 ns. During the first four of the equilibration periods, the solute atoms were harmonically restrained to their positions in the initial structures with force constants of 25 000, 2500, 250, and 25 kJ mol$^{-1}$ nm$^{-2}$. The temperature was kept constant using the weak-coupling algorithm50 with a relaxation or coupling time $\tau_T = 0.1$ ps. Solute and solvent, if present, were separately coupled to the heat bath. In the MD_aq simulations, the pressure was kept at 1 atm using the weak-coupling algorithm50 with a coupling time $\tau_p = 0.5$ ps and an isothermal compressibility $\kappa_T = 4.575 \times 10^{-4}$ (kJ mol$^{-1}$ nm$^{-2}$)$^{-1}$.

Following this equilibration procedure, the simulations were performed at a reference temperature of 300 K and, if in aqueous solution (MD_aq), a reference pressure of 1 atm. The center of mass motion of the system was removed after equilibration of the system.
After the equilibration of the protein in vacuo, in the MD simulations in vacuo (MD\textsubscript{vac}) the coupling to the heat bath was removed and translation of and rotation around the center of mass of the protein were not removed anymore in order to avoid perturbation of total energy conservation.

After the equilibration of the protein in vacuo, in the SD simulations in vacuo (SD\textsubscript{vac}) the temperature is maintained by the Langevin equations or thermostat, and by weak coupling to a heat bath ($\tau_T = 0.1$ ps), the latter in order to control the temperature of atoms that have a friction coefficient equal to zero, whose temperature is thus not controlled by the Langevin thermostat.

The required equilibration time before analysis of the trajectories depends mainly on the coupling between and the range of the interatomic forces, on the number of neighbor protein atoms at which solvent forces on solute atom are integrated forward in time. The atomic velocities are indicated by $\dot{v}_i$. The stochastic force $f_i^{\text{stoch}}(t)$ and the atomic friction coefficient $\gamma_i$ will only be sizable for protein atoms at the surface. Therefore, they are taken dependent on the number of neighbor atoms.\textsuperscript{33}

$$\gamma_i(t) = \gamma_{\text{solv}} \alpha_i(t)$$

with

$$\alpha_i(t) = \max\left(0, 1 - N_i^{\text{nb}}(t) / N_i^{\text{ref}}\right)$$

where $N_i^{\text{nb}}(t)$ denotes the number of nonhydrogen neighbor atoms of the protein atom $i$ within $0.3$ nm radius, and $N_i^{\text{ref}}$ was defined as an upper limit of six neighbor protein atoms at which solvent forces on solute atom $i$ are assumed to vanish. For water as solvent $\gamma_{\text{solv}} = 91$ ps\textsuperscript{-1}, and $\alpha_i(t)$ was updated every 1 ps during the simulation.\textsuperscript{33} The SD simulations were performed for $10$ ns with time steps of $2$, $4$, and $6$ fs and the constraint precisions $\text{tol}_{\text{DC}} = 10^{-4}$ or $10^{-5}$, $\text{tol}_{\text{BAC}} = 0.01^\circ$ or $0.001^\circ$, and $\text{tol}_{\text{BAC}} = 0.01^\circ$ or $0.001^\circ$. Translational motion of the center of mass of the system was removed every $2$ ps. Configurations were saved every $0.1$ ps and were used to analyze various dynamical properties as function of the type of constraints applied.

### 2.7 | SD simulation in vacuo without explicit water, but with frictional and stochastic forces

In stochastic dynamics (SD) simulation the Langevin equations of motion in Cartesian coordinates

$$m_i \frac{d^2 x_i}{dt^2} = -\frac{\partial V(x_1, x_2, ..., x_N)}{\partial x_i} + f_i^{\text{stoch}} - m_i \gamma_i \dot{v}_i, \quad i = 1, 2, ..., N_{\text{eff}}$$

are integrated forward in time. The atomic velocities are indicated by $\dot{v}_i$. The stochastic force $f_i^{\text{stoch}}(t)$ and the atomic friction coefficient $\gamma_i$ will only be sizable for protein atoms at the surface. Therefore, they are taken dependent on the number of neighbor atoms.\textsuperscript{33}
Table 3: Energy conservation in 100 ps MD simulations of the protein BPTI in vacuo (MD_vac) using three different sets of constraints, set BLC, set BADAC(H), and set ISDAC

| Constraints | tolLOC | tolBAC = tolLOC ('') | $\Delta t$ (fs) | $E_{\text{tot}}$ | $\Delta E_{\text{tot}}$ | $E_{\text{drift}}$ | $\Delta E_{\text{drift}}$ | $E_{\text{kin}}$ | $\Delta E_{\text{kin}}$ | $T$ (K) | $\Delta E_{\text{tot}}$/$\Delta E_{\text{kin}}$ | $\Delta E_{\text{drift}}$/$\Delta E_{\text{kin}}$ |
|-------------|--------|----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--------|-----------------------------|-----------------------------|
| BLC 10^{-5} - | 1 -1493 15 | -0.52 0.60 1471 45 | -0.32 44 298 0.34 0.14 |
| | 2 -1511 23 | -0.79 2.43 1466 44 | -0.32 43 297 0.52 0.056 |
| | 4 -1485 36 | -1.20 10.26 1466 43 | -0.50 41 297 0.84 0.253 |
| | 6.8 | * * * * * * * * * * |
| BLC + BADAC (H) 0.001 | 1 -2116 5 | -0.18 0.17 1152 40 | 0.11 40 308 0.13 0.004 |
| | 2 -2155 7 | -0.26 0.51 1114 39 | -0.24 38 298 0.19 0.013 |
| | 4 -2202 11 | -0.36 2.22 1116 38 | -0.08 38 298 0.28 0.058 |
| | 6 -2185 14 | -0.47 4.76 1106 36 | -0.27 36 295 0.40 0.134 |
| | 8 -2106 16 | -0.45 9.05 1136 35 | -0.05 35 303 0.46 0.260 |
| | 0.01 | 1 -2200 8 | -0.27 0.24 1107 39 | -0.17 39 295 0.20 0.006 |
| | 2 -2163 16 | -0.55 0.59 1128 39 | -0.23 38 301 0.41 0.016 |
| | 4 -2192 18 | -0.61 2.17 1110 37 | -0.26 37 296 0.47 0.059 |
| | 6 -2214 27 | -0.91 5.03 1107 38 | -0.50 35 296 0.70 0.142 |
| | 8 -2156 15 | -0.43 9.32 1136 34 | -0.02 34 303 0.46 0.276 |
| BLC + BADAC (H) + ISDAC 0.001 | 1 -2820 138 | -4.79 3.15 724 68 | -2.12 29 261 2.04 0.108 |
| | 2 -2913 164 | -5.66 7.63 680 77 | -2.49 27 245 2.13 0.284 |
| | 4 -2947 198 | -6.86 7.72 666 90 | -3.00 26 240 2.20 0.298 |
| | 8 -2993 219 | -7.58 13.47 639 103 | -3.46 25 230 2.13 0.534 |
| | 0.01 | 1 -1756 169 | -5.85 4.64 1339 84 | -2.58 40 271 2.00 0.117 |
| | 2 -1893 249 | -8.63 14.64 1281 121 | -3.98 37 259 2.06 0.394 |
| | 4 -2031 302 | -10.45 18.27 1216 140 | -4.70 35 246 2.16 0.529 |
| | 6.8 | * * * * * * * * * * |
| BLC 10^{-4} - | 1 -2166 41 | -1.42 0.57 1122 41 | -0.52 38 300 0.99 0.015 |
| | 2 -2331 93 | -3.23 1.90 1053 55 | -1.44 36 281 1.70 0.053 |
| | 4 -2379 137 | -4.73 5.02 1017 75 | -2.31 34 271 1.83 0.149 |
| | 6 -2423 164 | -5.68 7.95 995 82 | -2.61 31 266 2.01 0.253 |
| | 8 -2454 186 | -6.43 10.28 997 89 | -2.89 31 266 2.09 0.333 |
| | 0.1 | 1 -2226 39 | -1.34 1.10 1094 42 | -0.64 38 292 0.92 0.029 |
| | 2 -2363 95 | -3.28 0.89 1033 59 | -1.61 36 276 1.61 0.025 |
| | 4 -2441 138 | -4.79 4.40 985 75 | -2.33 33 263 1.85 0.134 |
| | 6 -2444 179 | -6.19 8.44 988 90 | -2.92 33 264 1.98 0.259 |
| | 8 -2489 179 | -6.21 10.40 977 88 | -2.89 29 261 2.03 0.358 |
| BLC + BADAC (H) + ISDAC 0.001 | 1 -2919 172 | -5.95 5.68 674 81 | -2.62 27 243 2.13 0.207 |
| | 4 -3017 239 | -8.25 17.33 625 116 | -3.92 27 225 2.06 0.654 |
| | 6 -3103 273 | -9.41 24.94 595 124 | -4.22 24 214 2.20 1.020 |
| | 8 -3122 303 | -10.45 28.48 589 140 | -4.78 24 212 2.17 1.195 |

Note: The bond-length constraints are imposed with a relative geometric precision of tolLOC, the bond-angle constraints with a precision of tolBAC, and the dihedral-angle constraints with a precision of tolLOC. *, no constraint solution, vectors orthogonal; #, no constraint solution, no convergence; Nonbonded interaction cut-off $R_{\text{c}} = R_{\text{in}}$ 100 nm (infinity); $\Delta t$, leap-frog integration time step; $E_{\text{tot}}$, total energy; $\Delta E_{\text{tot}}$, fluctuation of $E_{\text{tot}}$; $E_{\text{drift}}$, total energy drift; $\Delta E_{\text{drift}}$, fluctuation around $E_{\text{drift}}$; $E_{\text{kin}}$, kinetic energy; $\Delta E_{\text{kin}}$, fluctuation of $E_{\text{kin}}$; $E_{\text{kin}}$, kinetic energy drift; $\Delta E_{\text{kin}}$, fluctuation around $E_{\text{kin}}$; $T$, temperature. All values are averages calculated from the same number of trajectory structures separated by 0.02 ps. Energies in kJ mol$^{-1}$. Energy drifts in kJ mol$^{-1}$ ps$^{-1}$. 


where \(<...>_t\) indicates an average over time \(t\). The drift \(E^{\text{drift}}\) of an energy \(E\) can be defined as the slope of the line \(E^{\text{line}}(t)\) that is least-squares fitted to \(E(t)\) for a chosen period of time. The quantity

\[
\Delta E^{\text{drift}} = \left( \frac{1}{t} \left( E(t) - E^{\text{line}}(t) \right)^2 \right)^{1/2}
\]

represents the deviation of the actual energy from the line, \(E^{\text{line}}(t)\), representing the drift. \(\Delta E^{\text{drift}}\) represents the short-time-scale fluctuation of \(E\). This quantity may thus be better suited than \(\Delta E\) to evaluate the extent of total energy conservation while integrating the equations of motion.

The time evolution of structural features that would be sensitive to the way the bond-stretching forces are integrated or to whether bond-length constraints are applied, was examined in terms of autocorrelation functions and spectral densities of bond angles and of torsional angles. From a time series of a quantity \(Q(t)\), a normalized time correlation function,

\[
C_Q(t) = \frac{\langle Q(t) \cdot Q(t + \tau) \rangle}{\langle Q(t) \cdot Q(t) \rangle}
\]

was calculated using the Fast Fourier Transform technique. For these calculations, 25 ps towards the end of the simulations were repeated while saving configurations every 0.01 ps instead of 0.1 ps in order to obtain a finer resolution of the auto-correlation functions. When calculating the spectral density, only the first 2% of the auto-correlation function was used.

**FIGURE 1** Root-mean-square fluctuations (in degree) of the \(\phi\)-angles in the backbone of the protein BPTI as function of residue number in 10 ns MD simulations solvated in (SPC) water (MD\(_{\text{aq}}\)) and from 10 ns SD simulations in vacuo (SD\(_{\text{vac}}\)) using three different sets of constraints, set BLC, set BLC + BADAC(H), and set BLC + BADAC(H) + ISDAC, and for different time steps \(\Delta t\). Upper three panels: simulations MD\(_{\text{aq}}\). Lower two panels: simulations SD\(_{\text{vac}}\). Left panels: \(\text{tol}_{\text{DC}} = 10^{-5}\). Right panels: \(\text{tol}_{\text{DC}} = 10^{-4}\). \(\text{tol}_{\text{BAC}} = \text{tol}_{\text{DAC}} = 0.01^\circ\). Dotted lines: \(\Delta t = 2\) fs. Dashed lines: \(\Delta t = 4\) fs. Solid lines: \(\Delta t = 6\) fs.
3 | RESULTS

3.1 | MD simulation of a protein in vacuo to test conservation properties

In Table 3, the average total and kinetic energy and their fluctuations are shown for the protein in vacuo (MD_vac) using various time steps and different values of the geometric precision by which the bond-length (tolDC), bond-angle (tolBAC), and dihedral-angle (tolDAC) constraints are maintained. Constraining all bond lengths (BLC) in the protein with a relative precision of tolDC = 10^{-5}, the ratio $\Delta E_{\text{tot}}^{\text{drift}}/\Delta E_{\text{kin}}^{\text{drift}}$ of the fluctuation of the total energy around the total energy drift ($\Delta E_{\text{tot}}^{\text{drift}}$) and the fluctuation of the kinetic energy around the kinetic energy drift ($\Delta E_{\text{kin}}^{\text{drift}}$) changes from 0.014 for a time step $\Delta t = 1$ fs to 0.253 for a time step $\Delta t = 4$ fs. Using a lower precision of maintaining the bond-length constraints, tolDC = 10^{-4}, the ratio $\Delta E_{\text{tot}}^{\text{drift}}/\Delta E_{\text{kin}}^{\text{drift}}$ changes from 0.117 for a time step $\Delta t = 1$ fs to 0.529 for a time step $\Delta t = 4$ fs. So for these time steps, the precision of maintaining the bond-length constraints governs total energy conservation. Yet, using tolDC = 10^{-5} and $\Delta t = 4$ fs, the ratio $\Delta E_{\text{tot}}^{\text{drift}}/\Delta E_{\text{kin}}^{\text{drift}}$ is larger, 0.253, than using tolDC = 10^{-4} and $\Delta t = 1$ fs. For larger time steps, the integration error dominates the constraint error. Requiring a $\Delta E_{\text{tot}}^{\text{drift}}/\Delta E_{\text{kin}}^{\text{drift}}$ ratio of about 1/20 for total energy conservation, use of tolDC = 10^{-5} and $\Delta t = 2$ fs leading to a ratio of 0.056 seems possible. We note that in it was found that tolDC = 10^{-4} and $\Delta t = 2$ fs would lead to sufficient energy conservation. Since the protein BPTI in it was modeled using only united atoms, that is, without explicit hydrogens, the higher-frequency bond-length motions of the light hydrogen atoms were missing in the simulation, which allowed a lower precision for the bond-length constraints to be used for a given total energy conservation value.

**FIGURE 2** Root-mean-square fluctuations (in degree) of the $\psi$-angles in the backbone of the protein BPTI as function of residue number in 10 ns MD simulations solvated in (SPC) water (MD_aq) and from 10 ns SD simulations in vacuo (SD_vac) using three different sets of constraints, set BLC, set BLC + BADAC(H), and set BLC + BADAC(H) + ISDAC, and for different time steps $\Delta t$. Upper three panels: simulations MD_aq. Lower two panels: simulations SD_vac. Left panels: tolDC = 10^{-5}. Right panels: tolDC = 10^{-4}. tolBAC = tolDAC = 0.01. Dotted lines: $\Delta t = 2$ fs. Dashed lines: $\Delta t = 4$ fs. Solid lines: $\Delta t = 6$ fs.
When also constraining the relative positions of the hydrogen atoms, using constraint set BADAC(H) in addition to set BLC, the ratio $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ becomes much smaller. Using a high precision maintaining the constraints, $t_{\text{tol DC}} = 10^{-5}$ and $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.001^\circ$, the ratio $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ changes from 0.004 for a time step $\Delta t = 1$ fs to 0.260 for a time step $\Delta t = 8$ fs. Lowering the precision of the bond-angle and dihedral-angle constraints to $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.01^\circ$, the ratio $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ becomes only slightly larger comparing the same time step sizes, except for the small time step $\Delta t = 1$ fs. Lowering the precision of the bond-length constraints from $t_{\text{tol DC}} = 10^{-5}$ to $t_{\text{tol DC}} = 10^{-4}$, leads, as expected, to larger $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ values, in particular for the smaller time steps. Interestingly, the additional use of a lower precision $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.01^\circ$ instead of 0.001$^\circ$ leads to a lower ratio $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ for $\Delta t = 2$ and 4 fs, and to a comparable ratio for $\Delta t = 6$ fs. Yet using $t_{\text{tol DC}} = 10^{-4}$, the time step should not be larger than $\Delta t = 2$ fs, with a $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ ratio of 0.053 or 0.025 for $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.001^\circ$ or 0.01$^\circ$, respectively, because using $\Delta t = 4$ fs, the ratio becomes 0.149 or 0.134.

When also constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein, using constraint set ISDAC in addition to the sets BADAC(H) and BLC, the ratio $\Delta E_{\text{tot drift}}/\Delta E_{\text{kin drift}}$ becomes rather large, ranging from 0.108 to 1.195, for all time steps and constraint precision values $t_{\text{tol DC}}, t_{\text{tol BAC}}$ and $t_{\text{tol DAC}}$ investigated. For $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.001^\circ$ and $\Delta t = 1$–8 fs and for $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.01^\circ$ and $\Delta t = 1$ fs the algorithms to maintain the various constraints do not even converge. This is due to the large number of constraints that involve many of the same atoms and thus the

**FIGURE 3** Auto-correlation function (left panels) and spectral density (right panels) of the bond angle $\theta$(N-CA-C) of residue Phe 22 in the backbone of the protein BPTI in 10 ns MD simulations solvated in (SPC) water ($MD_{\text{aq}}$) and from 10 ns SD simulations in vacuo ($SD_{\text{vac}}$) using three different sets of constraints, set BLC, set BLC + BADAC(H), and set BLC + BADAC(H) + ISDAC, and for different time steps $\Delta t$. Upper three panels: simulations $MD_{\text{aq}}$. Lower two panels: simulations $SD_{\text{vac}}$. Dotted lines: $\Delta t = 2$ fs. Dashed lines: $\Delta t = 4$ fs. Solid lines: $\Delta t = 6$ fs. $t_{\text{tol DC}} = 10^{-5}$, $t_{\text{tol BAC}} = t_{\text{tol DAC}} = 0.01^\circ$. Configurations from 25 ps towards the end of the simulations, separated by 0.01 ps were used to calculate the auto-correlation functions and only the first 2% of the auto-correlation function was used to calculate the spectral density.
algorithms have trouble to find Lagrange multiplier values that make the molecular structure satisfy all constraints to within the specified precision. So the use of improper dihedral-angle and stiff proper (torsional) dihedral-angle constraints in the protein does not allow an increase of the time step in a MD simulation using Cartesian coordinates.

3.2 | MD simulation of a protein in aqueous solution to test dynamical protein properties

Using only bond-length constraints (set BLC), simulations in aqueous solution fail at $\Delta t = 4$ and 6 fs, because vectors become orthogonal during the execution of the SHAKE algorithm, an indication that disruptive forces occur. In simulations with constraint set ISDAC with $\Delta t = 2$ fs SHAKE does not converge.

Figures 1 and 2 (upper part) show the root-mean-square fluctuations of the backbone $\varphi$ and $\psi$ torsional angles as function of residue number applying the different sets of constraints and time steps $\Delta t$. The larger peaks are due to the occurrence of relatively rare (on the nanosecond time scale) torsional-angle transitions over relatively low barriers separating the different minima of the torsional-angle potential-energy terms in the force field used. For example, in the simulation with constraint set BLC, $\text{tol}_{DC} = 10^{-4}$ and $\text{tol}_{BAC} = \text{tol}_{DAC} = 0.01^\circ$, with $\Delta t = 2$ fs, the peptide plane between residues 39 and 40 changes orientation which induces a correlated change in $\psi(39)$ and $\varphi(40)$.

The application of constraints when integrating the equations of motion would primarily affect the motions along the nonconstrained degrees of freedom that are close or adjacent to the constrained ones. The influence of the different combinations of the three sets of

**FIGURE 4** Auto-correlation function (left panels) and spectral density (right panels) of the torsional angle $\psi$(N-CA-C-N) of residue Arg 17 in the backbone of the protein BPTI in 10 ns MD simulations solvated in (SPC) water (MD\textsubscript{aq}) and from 10 ns SD simulations in vacuo (SD\textsubscript{vac}) using three different sets of constraints, set BLC, set BLC + BADAC(H), and set BLC + BADAC(H) + ISDAC, and for different time steps $\Delta t$. Upper three panels: simulations MD\textsubscript{aq}. Lower two panels: simulations SD\textsubscript{vac}. Dotted lines: $\Delta t = 2$ fs. Dashed lines: $\Delta t = 4$ fs. Solid lines: $\Delta t = 6$ fs. $\text{tol}_{DC} = 10^{-5}$, $\text{tol}_{BAC} = \text{tol}_{DAC} = 0.01^\circ$. Configurations from 25 ps towards the end of the simulations, separated by 0.01 ps were used to calculate the auto-correlation functions and only the first 2% of the auto-correlation function was used to calculate the spectral density.
constraints can be inferred from Figure 3 (upper part), which shows the auto-correlation function and spectral density of the bond angle \( \theta(N-CA-C) \) of residue Phe 22 in the backbone of the protein for the different sets of constraints and time steps \( \Delta t \). The curves resulting from the application of the constraint sets \( BLC \) and \( BLC + BADAC(H) \) are almost identical. When also constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein, using constraint set \( ISDAC \) in addition to the sets \( BADAC(H) \) and \( BLC \), slightly larger differences are observed.

Figures 4 and 5 (upper part) show the auto-correlation function and spectral density of the torsional angles \( \psi(N-CA-C-N) \) of residue Arg 17 and \( \phi(C-N-CA-C) \) of residue Ile 18 in the backbone of the protein applying the different sets of constraints and time steps \( \Delta t \). Using the constraint sets \( BLC \) and set \( BLC + BADAC(H) \), the spectral densities are rather similar, while the auto-correlation functions show differences in the longer time (beyond 0.2 ps) correlation. This is due to torsional-angle transitions occurring rarely on the simulated time scale. The difference of the angle at time \( t \) with its average is much larger when a transition occurs than when this is not the case. When a transition occurs, the difference of the angle with its average is thus only slowly reduced, leading to a slow decay of the auto-correlation function. In case there is no transition, the difference of the angle with its average is much smaller and changes much more rapidly, leading to a much faster decay of the auto-correlation function. The effect of relatively rare torsional-angle transitions on the auto-correlation function is even more prominent for side-chain torsional angles, as is illustrated in Figure 6 for the side-chain torsional angle \( \chi_2(CA-CB-CG-CD) \) of residue Arg 39. When also constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein, using constraint set \( ISDAC \) in addition to the sets \( BADAC(H) \) and \( BLC \),
the peak in the spectral densities of the torsional angles $\psi$(N-CA-C-N) of residue Arg 17 and $\varphi$(C-N-CA-C) of residue Ile 18 at about 9 ps$^{-1}$ is gone.

In summary, when comparing the motions along nonconstrained degrees of freedom in the MD simulations of the protein in aqueous solution using the different combinations of the two sets of constraints, set BLC and set BADAC(H), no large differences are observed. This indicates that the motions along these constrained degrees of freedom are not strongly coupled to the motions along the other degrees of freedom of the protein. This allows constraining the relative positions of the hydrogen atoms in the protein, using constraint set BADAC(H) in addition to set BLC, and a time step $\Delta t = 4$ fs. When also constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein, using constraint set ISDAC in addition to the sets BADAC(H) and BLC, larger variations in mobility are observed between the different time step sizes.

In a study$^{53}$ similar to the current one it was observed that the presence of many water molecules, as in liquid water, limits the size of the MD time step to about 2 fs.

### 3.3 SD simulation of a protein in vacuo to test dynamical protein properties

In a stochastic dynamics simulation of a protein, the influence of the solvent molecules on the protein structure and dynamics is modeled using a mean-field approximation by stochastic and frictional forces acting on the protein atoms, see Equations (5–7). Due to the absence of specific interactions, such as hydrogen bonding, between protein
atoms and water molecules, the protein structure and mobility may differ from that in a simulation with explicit water molecules, see for example.\textsuperscript{33,34} This can be addressed by adding an additional force-field term, that in a mean-field manner approximates the influence of the solvent molecules. Since in the present study the focus is on the influence of constraining particular degrees of freedom on the dynamics and mobility of the protein atoms, such mean-field force-field terms were not used in order to minimize the changes in the force field between the three simulation types, MD in vacuo (MD\textsubscript{vac}), MD in aqueous solution (MD\textsubscript{aq}) and SD in vacuo (SD\textsubscript{vac}).

Figures 1 and 2 (lower part) show the root-mean-square fluctuations of the backbone $\phi$ and $\psi$ torsional angles as function of residue number applying the different sets of constraints and time steps $\Delta t$. Constraining all bond lengths (BLC) in the protein, the simulations fail to converge for $\text{tol}_{/C0} = 10^{-5}$ and $\Delta t = 6$ fs and for $\text{tol}_{/C0} = 10^{-4}$ and $\Delta t = 4$ and 6 fs. The torsional-angle fluctuations are more or less comparable, the differences being due to differences in torsional-angle transitions occurring during the simulations. All SD simulations using constraint set ISDAC fail due to vectors becoming orthogonal in the SHAKE procedure.

When also constraining the relative positions of the hydrogen atoms, using constraint set BADAC(H) in addition to set BLC, the larger time steps can be used. Constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein, using constraint set ISDAC in addition to the sets BADAC(H) and BLC in the SD simulations, no convergence could be obtained.

Figure 3 (lower part) shows the auto-correlation function and spectral density of the bond angle $\phi$(N-C-A-C) of residue Phe 22 in the backbone of the protein for the different sets of constraints and time steps $\Delta t$. The curves resulting from the application of the constraint sets BLC and BLC + BADAC(H), are similar. For the large time steps $\Delta t = 4$ and 6 fs, the peak at about 9 ps$^{-1}$ is more pronounced when constraining the relative hydrogen positions. The auto-correlation functions and spectral densities do resemble those for the MD simulations in aqueous solution (MD\textsubscript{aq}).

Figures 4 and 5 (lower part) show the auto-correlation function and spectral density of the torsional angles $\psi$(N-C-A-C) of residue Arg 17 and $\phi$(C-N-C-A-C) of residue Ile 18 in the backbone of the protein applying the different sets of constraints and time steps $\Delta t$. Using the constraint sets BLC and set BLC + BADAC(H), the spectral densities are rather similar, while the auto-correlation functions show differences in the longer time (beyond 0.2 ps) correlation. This is due to torsional-angle transitions occurring rarely on the simulated time scale. Again, the auto-correlation functions and spectral densities do resemble those obtained from the MD simulations in aqueous solution (MD\textsubscript{aq}).

In summary, when comparing the motions along nonconstrained degrees of freedom in the SD simulations of the protein in vacuo using the two sets of constraints BLC and BLC + BADAC(H), no large differences are observed. This indicates that the motions along these constrained degrees of freedom are not strongly coupled to the motions along the other degrees of freedom of the protein. This allows constraining the relative positions of the hydrogen atoms in the protein, using constraint set BADAC(H) in addition to set BLC, and a time step $\Delta t = 4$ fs.

\section{Conclusions}

The application of bond-angle and dihedral-angle constraints, in addition to bond-length constraints, in molecular (MD) and stochastic (SD) dynamics simulations of a protein, bovine pancreatic trypsin inhibitor (BPTI), is investigated with an eye to lengthening the time step by which the classical equations of motion are integrated forward in time. Using Newton’s equations of motion the extent of conservation of the total energy will be determined by the numerical integration algorithm (the leap-frog algorithm), the precision of the algorithms to impose the bond-length ($\text{tol}_{/C0}$), bond-angle ($\text{tol}_{/BAC}$) and dihedral-angle ($\text{tol}_{/BADAC}$) constraints, and the size of the MD time step $\Delta t$. For the MD simulations of the protein solvated in water and for the SD simulations of the protein in vacuo it is investigated whether dynamical properties of selected nonconstrained degrees of freedom are affected by the application of the various types of constraints.

Using total energy conservation as criterion, constraining all bond lengths in the protein with a relative geometric precision of $\text{tol}_{/C0} = 10^{-5}$ allows for a time step $\Delta t = 2$ fs. In one of the earliest protein simulation studies,\textsuperscript{8} using a simple protein model containing no hydrogen atoms, only united atoms, it was found that a time step of $\Delta t = 2$ fs could be used when the bond-length constraints between the united atoms were maintained with a precision of $\text{tol}_{/C0} = 10^{-4}$. Since in current models of proteins the light hydrogen atoms are explicitly treated, their relatively fast motion requires a higher precision, $\text{tol}_{/C0} = 10^{-5}$, when constraining the bond lengths in a protein.

Constraining the relative positions of the hydrogen atoms, in addition to constraining all bond lengths in the protein, allows for a lengthening of the time step by a factor two to $\Delta t = 4$ fs, without much distortion of the dynamics of the protein.

The computational effort of the evaluation of the bond-stretching and bond-angle bending forces at a number of small time steps $\Delta t$ is comparable to that of maintaining the bond-length (and bond-angle) constraints at the larger time step $\Delta t$.

Constraining the improper dihedral angles and the stiff proper (torsional) dihedral angles in the protein, in addition to constraining all bond lengths and the relative positions of the hydrogen atoms, does not allow for a lengthening of the time step in a MD or SD simulation using Cartesian coordinates. This is due to the large number of constraints that involve many of the same atoms and thus the algorithms that maintain the constraints have trouble to find Lagrange multiplier values that make the molecular structure satisfy all constraints to within the specified precision.

When simulating a protein solvated in water, it may not be possible to use a 4 fs time step while constraining the relative positions of the hydrogen atoms, because the relatively fast librational motions of the rigid water molecules present may not allow for a time step longer than 2 fs. Furthermore, in simulations including water in a periodic box the nonbonded interaction cut-off is the dominant factor for
nonconservation of energy and leads to changes in some dynamic water properties at time steps higher than 2 fs.33

Yet, when performing SD simulation in vacuo, including a proper potential of mean force mimicking the influence of the solvent upon the protein motion, a time step of 4 fs can be used when constraining the relative positions of the hydrogen atoms.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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