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Lennard-Jones type pair-potential method for coarse-grained lipid bilayer membrane simulations in LAMMPS*



S.-P. Fu^{a,*}, Z. Peng^b, H. Yuan^c, R. Kfoury^c, Y.-N. Young^a

^a Department of Mathematical Sciences, New Jersey Institute of Technology, Newark, NJ 07102, USA

^b Department of Aerospace and Mechanical Engineering, University of Notre Dame, Notre Dame, IN 46556, USA

^c Department of Mechanical, Industrial & Systems Engineering, University of Rhode Island, Kingston, RI 02881, USA

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ABSTRACT

Lipid bilayer membranes have been extensively studied by coarse-grained molecular dynamics simulations. Numerical efficiencies have been reported in the cases of aggressive coarse-graining, where several lipids are coarse-grained into a particle of size $4 \sim 6$ nm so that there is only one particle in the thickness direction. Yuan et al. proposed a pair-potential between these one-particle-thick coarse-grained lipid particles to capture the mechanical properties of a lipid bilayer membrane, such as gel-fluid-gas phase transitions of lipids, diffusion, and bending rigidity Yuan et al. (2010). In this work we implement such an interaction potential in LAMMPS to simulate large-scale lipid systems such as a giant unilamellar vesicle (GUV) and red blood cells (RBCs). We also consider the effect of cytoskeleton on the lipid membrane dynamics as a model for RBC dynamics, and incorporate coarse-grained water molecules to account for hydrodynamic interactions. The interaction between the coarse-grained water molecules (explicit solvent molecules) is modeled as a Lennard-Jones (L-J) potential. To demonstrate that the proposed methods do capture the observed dynamics of vesicles and RBCs, we focus on two sets of LAMMPS simulations: 1. Vesicle shape transitions with enclosed volume; 2. RBC shape transitions with different enclosed volume. Finally utilizing the parallel computing capability in LAMMPS, we provide some timing results for parallel coarse-grained simulations to illustrate that it is possible to use LAMMPS to simulate large-scale realistic complex biological membranes for more than 1 ms.

Program summary

Program Title: fluidmembrane

Program Files doi: http://dx.doi.org/10.17632/4v53nkv5hc.1

Licensing provisions: GNU GPLv3

Programming language: C++.

Nature of problem:

A pair-potential function for simulating the shape transition of fluid vesicles and the resting shapes of red blood cells.

Program Solution method:

Nosé-Hoover thermostat and Berendsen pressure coupling algorithms.

External routines/libraries: LAMMPS

Subprograms used: create_rbc_with_water (MATLAB), bond_harmonic1 (C++), in_example (LAMMPS input script).

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- * Corresponding author.
- *E-mail address*: sf47@njit.edu (S.-P. Fu). http://dx.doi.org/10.1016/j.cpc.2016.09.018 0010-4655/© 2016 Elsevier B.V. All rights reserved.

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1. Introduction

In recent years, great progress has been made to understand the dynamics of vesicles (self-enclosing lipid bilayer membranes) and red blood cells (RBCs) in aqueous solutions due to their relevance in a wide range of fields such as biology, biophysics, and biomedical engineering. A main component of the vesicle and RBC membranes is the amphiphilic lipid molecules, which self-assemble to form liposomes (vesicles) or micelles. In a viscous fluid flow, the vesicle may deform due to the balance between viscous stress, bending resistance and tension forces in the membrane. In this work we focus on pure lipid bilayer membrane and neglect the effects of multiple lipid species and different transmembrane proteins on the lipid bilayer membrane. For a pure lipid bilayer membrane, the equilibrium shapes of vesicles immersed in fluid have been widely studied in continuum modeling and coarse-grained molecular dynamics (CGMD) modeling. In the continuum framework, the dynamics and equilibrium shape of a lipid bilayer membrane is governed by the Helfrich free energy that consists of mean, Gaussian and spontaneous curvatures of the membrane [1]. The total membrane energy is integrated over the surface Ω as

$$E = \int_{\Omega} \left[\gamma + \frac{B}{2} (c_1 + c_2 - c_0)^2 + \bar{\kappa} c_1 c_2 \right] dA, \tag{1}$$

where { c_1 , c_2 } are the principle curvatures, γ is the surface tension, B is the bending rigidity, $\bar{\kappa}$ is the saddle-splay modulus and c_0 is the spontaneous curvature. Without membrane tension ($\gamma = 0$) and saddle-splay energy ($\bar{\kappa} = 0$), Eq. (1) is reduced to the classical Helfrich–Canham energy which consists of only the bending rigidity and spontaneous curvature:

$$E = \int_{\Omega} \frac{B}{2} (c_1 + c_2 - c_0)^2 dA.$$
 (2)

Continuum modeling has successfully reproduced vesicle and RBC dynamics in a fluid flow [2], even though several physical properties of the lipid bilayer membrane (such as membrane diffusivity and temperature-dependent bending rigidity) have to be assumed in the continuum framework. In the aggressive coarse-graining, the lipid bilayer membrane is modeled as a one-particle-thick monolayer of coarse-grained lipid particles, with each particle containing many lipids. By specifying the potential for particle interaction, the lipid properties of hydrophilic heads and hydrophobic tails can be preserved [3]. Hence, a characteristic length scale of oneparticle-thick CGMD membrane model is often chosen as the thickness (4-5 nm) of lipid bilayer membrane. In this work we focus on the meshfree CGMD membrane model, first introduced by Drouffe et al. in early 1990s [4]. They showed that their simulation method can well predict the self-assembly property of lipid bilayer membrane. By controlling the model parameters in one-particle-thick CGMD membrane model, Yuan et al. showed that the various lipid phases (gel, fluid and gas) and the physically reasonable lipid diffusivity can be achieved [3]. With the development of LAMMPS and advancement of large-scale parallel computing, long-time simulations are now more achievable for examining the dynamics of lipid bilayer membranes in aqueous solutions. In this work we adopt CGMD and implement the pair-potential function for the coarsegrained lipid particles in LAMMPS for numerical investigation of vesicle and RBC dynamics.

To fully understand the dynamics of a lipid bilayer membrane with spontaneous curvature, two approaches are introduced in the particle based MD simulations: (1) Pair-potential for fluid lipid membrane which involves membrane-solvent, fluid membrane network and solvent–solvent interactions [3,5–7]. (2) Local multibody curvature energy which consists of a local curvature potential based on aplanarity, excluded volume (as a repulsive) potential and

an attractive potential that depends on the local particle density [8,9]. Both approaches have their particular advantages in numerical investigation, and results show that they are able to reproduce the self-assembly property of a fluid-phase lipid membrane. In this work we implement the pair-potential function for CG lipid bilayer membrane in LAMMPS. We will illustrate how to simulate the membrane dynamics with either explicit or implicit solvent in LAMMPS to study the membrane property and dynamic shape transitions of vesicles and red blood cells. Two specific applications to the biological systems using LAMMPS are presented in this paper:

1. Vesicle shape transitions

Seifert et al. calculated the phase diagram of vesicle shape transitions using the Helfrich free energy (with a spontaneous curvature) described above, and provided detailed theoretical insight to the vesicle equilibrium shapes by comparing with experiments [10,11]. We will illustrate how to use LAMMPS to model the vesicle shape transition due to volume reduction. In the one-particle-thick CGMD membrane model, we will include coarse-grained water molecules (explicit solvent) to account for hydrodynamic interactions.

2. Resting shapes of RBCs

We extend the CGMD modeling of a vesicle to a RBC, where the surface structure is a lipid bilayer membrane coupled with a layer of cytoskeleton network underneath. Laboratory experiments show that RBC can form stomatocyte, discocyte and echinocyte minimum-energy shapes. In early 2000s, Lim et al. introduced the mechanical theory for predicting the stable RBC shapes involving area difference between outer and inner leaflets of RBCs. Lim et al. adopted area-difference-elasticity model (ADE) which describes the free energy using spontaneous curvature and geometrical area difference of RBCs to reproduce the RBC shapes under cases of reduce relaxed area difference and compared the simulation results of RBC shapes with experiments [12].

In the past, the hydrodynamics of a RBC in a fluid flow has been simulated by using finite element method (FEM), immersed boundary method (IB), dissipative particle dynamics (DPD) [13– 15] and Langevin dynamics. In this work we adopt Nosé–Hoover algorithms in CGMD and compare our numerical results with previous results from continuum model simulations.

This paper is organized as follows: In Section 2 we introduce the new pair-potential model for fluid membrane and numerical integration scheme for MD simulations in LAMMPS. In Section 3 we discuss the methodology of accounting for the hydrodynamic interaction in this work. In Section 4 we provide the details of setting up the case studies in LAMMPS. In Section 5 we analyze the membrane property such as in-plane lipid diffusivity and bending rigidity by simulating a square membrane patch in 3D box. In Section 6 we demonstrate the dynamics of vesicle and RBC from LAMMPS simulations and compare with previous simulation results [2,7] where the solvent and internal fluid are coarsegrained. Finally, Section 7 includes a short conclusion and remark for LAMMPS users.

2. Model descriptions

2.1. Coarse-Grained (CG) modeling

The advantage of using coarse-grained modeling is to reduce the computational cost. Without losing physical properties of lipid bilayer membrane, characteristic length scale in CG modeling can be much larger than atomistic sizes and simulations can be performed for a much longer time. Fig. 1 shows that lipid bilayer membrane can be represented as: (a) 1 bead for lipid head and rigid rod for lipid tail [16]; (b) 1 bead for lipid head and 2 beads



Fig. 1. Various CG membrane models: (a) 1 bead for lipid head and rod for lipid tail; (b) 1 bead for lipid head and 2 beads for lipid tail; (c) 1 bead for a collection of lipids without mesh. Source: panel (a) and (b) are reprinted from [17]; panel (c) is reprinted from [8].



Fig. 2. Schematics of normal vectors, angular parameters for the particle pair $\{\mathbf{r}_i, \mathbf{r}_j\}$ and relationship between angle parameter θ_0 and spontaneous curvature c_0 where d_0 is the average interparticle distance.

for lipid tail [17]; (c) 1 bead for a collection of lipids with the bead diameter as the thickness of lipid bilayer membrane [8,9]. For all LAMMPS simulations in this work, we adopt the coarse-graining in panel (c), the one-particle-thick meshless model where the characteristic length is the diameter of coarse-grained particle (lipid bilayer membrane thickness).

2.2. Pair-potential model for membrane

In this work we implement in LAMMPS the lipid–lipid interaction potential function for CGMD simulations of a lipid bilayer membrane. Developed by Yuan et al., the interaction potential between coarse-grained lipid particles is constructed to account for the head–head, tail–tail and head–tail interactions between the coarse-grained lipid mesoscopic molecules [3,7]. Fig. 2 shows the schematic of inter-particle interactions, angular parameters and the approximation of spontaneous curvature c_0 . d_0 is the average inter-particle distance and the dimensionless spontaneous curvature is $\tilde{c_0} = R_0 c_0$ where R_0 is the radius of spherical body. $\{\theta_i, \theta_j\}$ indicate the orientations of particle pair $\{\mathbf{r_i}, \mathbf{r_j}\}$.

Denoting the position of *i*th CG particle as \mathbf{r}_i , for each pair of particles $\{\mathbf{r}_i, \mathbf{r}_j\}$, we only consider the repulsive potential $u_R(r)$ and attractive potential $u_A(r)$ which are given by the following formulas

$$u_{R}(r) = \epsilon \left[\left(\frac{r_{min}}{r} \right)^{4} - 2 \left(\frac{r_{min}}{r} \right)^{2} \right]$$

$$u_{A}(r) = -\epsilon \cos^{2\zeta} \left(\frac{\pi}{2} \frac{(r - r_{min})}{(r_{c} - r_{min})} \right),$$
(3)

with $r = |\mathbf{r}_{ij}| \equiv |\mathbf{r}_i - \mathbf{r}_j|$. The exponent ζ controls the slope of the attractive branch and r_c is the cutoff radius. r_{min} is the distance which minimizes the potential energy $u_A(r)$ and $r_{min} = \sqrt[6]{2}\sigma$,

where σ is the length unit. ϵ is the energy unit which we set $k_B T = 0.23\epsilon$ for numerical simulations in Section 6.

We then define an angular function $\phi(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j)$ which depends on the relative orientation between particle pair \mathbf{r}_i and \mathbf{r}_j :

$$\phi(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j) = 1 + \mu(a(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j) - 1)$$

$$a(\hat{\mathbf{r}}_{ii}, \mathbf{n}_i, \mathbf{n}_j) = (\mathbf{n}_i \times \hat{\mathbf{r}}_{ij}) \cdot (\mathbf{n}_i \times \hat{\mathbf{r}}_{ij}) + \sin\theta_0(\mathbf{n}_i - \mathbf{n}_i) \cdot \hat{\mathbf{r}}_{ij}$$
(4)

$$\mathbf{r}_{ij}, \mathbf{n}_i, \mathbf{n}_j) = (\mathbf{n}_i \times \mathbf{r}_{ij}) \cdot (\mathbf{n}_j \times \mathbf{r}_{ij}) + \sin \theta_0 (\mathbf{n}_i - \mathbf{n}_j) \cdot \mathbf{r}_{ij} - \sin^2 \theta_0$$
(5)

where $\hat{\mathbf{r}}_{ij} = \mathbf{r}_{ij}/r$, μ is the parameter related to bending rigidity and θ_0 is the parameter related to the spontaneous curvature. The pairinteraction potential U of each pair of particles { \mathbf{r}_i , \mathbf{r}_j } is expressed in terms of the angular function ϕ , $u_R(r)$, and $u_A(r)$ as

$$U(\mathbf{r}_{ij}, \mathbf{n}_i, \mathbf{n}_j) = \begin{cases} u_R(r) + [1 - \phi(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j)]\epsilon, & r < r_{min} \\ u_A(r)\phi(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j), & r_{min} < r < r_c. \end{cases}$$
(6)

Fig. 3(a) shows the variation of $a(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j)$ with $\{\theta_i, \theta_j\}$, and Fig. 3(b) shows the dependence of the attractive component of the potential $U(\mathbf{r}_{ij}, \mathbf{n}_i, \mathbf{n}_j)$ on parameter ζ . From the formulas above, the simplest case is when the normal vectors $\{\mathbf{n}_i, \mathbf{n}_j\}$ are parallel, which gives a = 1 and $\phi = 1$. Finally, after implementation is completed in LAMMPS, we name this new pair-potential function as

pair_fluidmembrane

and this package includes one main script and one header file. To call this pair interaction function in LAMMPS, the input commands are given as follows:

```
pair_style hybrid lj/cut 3.6 fluidmembrane 2.6
pair_coeff 1*2 1*2 fluidmembrane 1.0 1.0 2.6 4 3 0
```

where we give the parameters for global cut-off lengths in the pair-potential. The above LAMMPS commands can also be used to call the Lennard-Jones potential, which is used to model the interaction with explicit solvents in Section 6. The sequence of parameters for "fluidmembrane" pair function are given by: $\epsilon \sigma r_{cut} \zeta \mu \sin \theta_0$.

2.3. Cytoskeleton

The cytoplasmic membrane of a RBC is coupled to a cytoskeleton network. Within the coarse-grained formulation, the cytoskeleton network is modeled as a polymeric network that contains three basic types of coarse-grained particles: (1) Junction complexes (actin protofilament and protein band 4.1) are located at the end of spectrin tetramers, (2) Spectrin tetramers (composed of consecutive bonded beads), and (3) Ankyrin proteins located in middle of spectrin beads which connect network



Fig. 3. (a) Surface plot for the values of $a(\hat{\mathbf{r}}_{ij}, \mathbf{n}_i, \mathbf{n}_j)$ versus corresponding $\{\theta_i, \theta_j\}$; (b) Dependence of potential energy on angular parameter θ_0 when the model parameter $\zeta = 4.0$.



Fig. 4. Cross-section snapshot of cubical simulation box for coarse-grained RBC simulation in LAMMPS with periodic boundary condition; 7 types of particles are shown in this image: lipid membrane (blue), transmembrane protein (pink), junction complexes (dark purple), spectrin tetramers (lime), ankyrin (light purple), internal water (brown) and external water (gray) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to transmembrane proteins. Since we focus on the RBC shape transition at the small deformation regime, we use harmonic springs to model the connectivity between the coarse-grained lipid bilayer membrane and the cytoskeleton network through binding with spectrin, ankyrin and other linking proteins.

The harmonic bond potential is given by Eq. (7):

$$E_{harmonic} = K(r_{ij} - r_0)^2, \tag{7}$$

where K is a constant and r_0 is the equilibrium distance of each bond. The number of coarse-grained cytoskeleton particles depends on the average distance between each pair of ankyrins. As the cytoskeleton network is enclosed by the lipid bilayer membrane, strong thermal fluctuations may cause nonphysical phenomenon in the CGMD simulations. For example, the coarsegrained cytoskeleton particles may move to the cell exterior. In our simulations precautions are made to prevent this from happening. In the initial configuration of the CGMD simulations of RBC, we put no water molecule between the membrane and cytoskeleton network.

Fig. 4 shows the initial configuration of a coarse-grained RBC and water molecules. Including internal water molecules and external water molecules, we have total 7 types of particles for simulations in LAMMPS.

2.4. Langevin dynamics

The dynamics of a coarse-grained macromolecule has been modeled by the Langevin equation. In the case of a coarse-grained lipid bilayer membrane, the Langevin equation with constant friction coefficient is often adopted:

$$m_i \frac{d^2 \mathbf{r}_i}{dt^2} = -\zeta \frac{d\mathbf{r}_i}{dt} + \mathbf{F}_i + \sqrt{2k_B T \zeta} \mathbf{W}_i, \tag{8}$$

where m_i is the mass of coarse-grained particle *i*, ζ is the friction coefficient and \mathbf{F}_i is the interparticle force. The coefficient of Wiener process \mathbf{W}_i connects the thermal fluctuations of the particles through hydrodynamic interactions. Thermal fluctuation effect is significant in this length scale following from fluctuation–dissipation theorem therefore the axisymmetric case is not considered in this work. In the absence of external driving forces, the covariance between the bead displacements satisfies the following relation

$$\langle \mathbf{W}_{i}(t) \rangle = 0, \quad \langle \mathbf{W}_{i}(t)\mathbf{W}_{j}(t') \rangle = 2k_{B}T\zeta\delta_{ij}\delta(t-t'). \tag{9}$$

Therefore, the magnitude of thermal fluctuation can be controlled by fixing the system temperature.

2.5. Nondimensionalization

For LAMMPS simulations, we nondimensionalize length, time and temperature units by following the scaling by specifying the LAMMPS unit style units lj:

$$l_s = \sigma$$
, where $x^* = \frac{x}{\sigma}$; $t_s = \tau$,
where $t^* = t\sqrt{\frac{\epsilon}{m\sigma^2}}$; $T^* = T\frac{k_B}{\epsilon}$,



Fig. 5. Lennard-Jones potential and force curves with $\sigma_{eq} = 2.7$ and $\epsilon = 1.0$. The blue dot represents the case when the minimum energy occurs at $r_{min}/\sigma_{eq} = 2^{1/6} \approx 1.12$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

where m is the mass of each CG membrane particle and T is the temperature. In our LAMMPS input, we set

mass bilayer 1.0

3. Hydrodynamic and interparticle interactions

In this work, the Lennard-Jones (L-J) potential is used for the interaction between coarse-grained lipid particles, coarse-grained cytoskeleton and water molecules. As water molecules may pass through the porous structures in the cytoskeleton, we need to take the membrane–water interactions into account. In addition, we also add L-J potentials for cytoskeleton network and membrane to prevent proteins from coming out of the RBC. The L-J potential is given by the following formula

$$E_{LJ} = 4\epsilon \left[\left(\frac{\sigma_{eq}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{eq}}{r_{ij}} \right)^{6} \right], \tag{10}$$

where r_{ij} is: (a) distance between two coarse-grained water molecules; (b) distance of each pair of coarse-grained cytoskeleton particles; (c) distance between membrane or cytoskeleton and water molecules. σ is the equilibrium length to the interactions. To implement these interactions in LAMMPS, we have the following commands in the input script:

pair_coeff 1*26*7 lj/cut	: 0.2	1.0
pair_coeff 1*2 3*5 lj/cut	0.2	1.0
pair_coeff 3*5 3*5 lj/cut	0.2	1.0
pair_coeff 3*5 6*7 lj/cut	0.2	1.0
pair_coeff 6*7 6*7 lj/cut	0.2	2.7

where atom type 1–2 are membrane components, type 3–5 form cytoskeleton network and atom type 6–7 are internal and external water molecules.

Fig. 5 shows one example when $\sigma_{eq} = 2.7$, $\epsilon = 1.0$ case. For water-water interactions, a smaller value of σ_{eq} implies a smaller effective volume occupied by the coarse-grained water molecules. Instead of changing the number of coarse-grained water molecules to adjust the interior RBC/vesicle volume, we adjust σ_{eq} for the coarse-grained internal water molecules to control the volume in RBC. This approach is advantageous as it gives the desired interior volume in LAMMPS simulations.

4. Implementation in LAMMPS

The initial configuration for LAMMPS simulations is generated by a MATLAB code. For vesicle simulations it includes membrane, internal water molecules and external water molecules. In the case of CGMD RBC simulations, an addition of cytoskeleton network Table 1

The atom styles used in current numerical simulations.

Ellipsoid	Atom-ID atom-type ellipsoidflag density x y z
Peri	Atom-ID atom-type volume density x y z
Molecular	Atom-ID molecule-ID atom-type x y z
Hybrid	Atom-ID atom-type x y z sub-style1 sub-style2 sub-style3

is generated. In LAMMPS we specify multiple atom styles using hybrid function and call "ellipsoid", "molecular" and "peri". Here we provide the LAMMPS command in the script:

```
atom_style hybrid ellipsoid peri molecular
```

"peri" is for extracting the initial configurations (x_0 in LAMMPS) of all coarse-grained particles including bilayer membrane and cytoskeleton network [18]. The data format provided below is for satisfying the hybrid atom style listed in Table 1 from LAMMPS guidelines:

```
atom-ID atom-type x y z ellipsoidflag
density volume density molecule-ID
```

4.1. Modified harmonic bond function

The water volume enclosed in the lipid bilayer membrane is controlled by adjusting the effective radius of the coarse-grained water molecules. For the case of a RBC, it is important to ensure that RBC cytoskeleton is stress-free in the initial configuration so we can conduct comparison with previous results in the literature. One way to ensure a stress-free RBC cytoskeleton is by modifying the equilibrium bond length in Eq. (7) so that initially the harmonic bond energy is zero for the cytoskeleton.

Thus, we modified the harmonic bond in LAMMPS by calling initial configuration x_0 and calculate the bond length $l_0(\mathbf{r}_i, \mathbf{r}_j)$ between each pair of particles at the beginning of simulation. Different from the harmonic bond function in LAMMPS where the bond length r_0 is a constant in Eq. (7), the modified harmonic bond energy is now

$$\tilde{E}_{harmonic} = K(r_{ii} - l_0(\mathbf{r}_i, \mathbf{r}_i))^2,$$
(11)

where $l_0 = |x_0, ij|$ is the initial length of the bond. This slight modification helps us achieve a stress-free configuration for the cytoskeleton before we reduce the RBC volume.

We first show the LAMMPS command which is made for the specific bond style: bond_style harmonic1 The following lines are the codes we modified from

bond_harmonic.cpp

and create a new C++ source code and header file with the name

```
bond_harmonic1.cpp
bond harmonic1.h
```

The following lines are the code which calculates the bond lengths l_0 in Eq. (11):

```
double **x0 = atom \rightarrow x0;

double **x = atom \rightarrow x;

delx0 = x0[i1][0] - x0[i2][0];

dely0 = x0[i1][1] - x0[i2][1];

delz0 = x0[i1][2] - x0[i2][2];

delx = x[i1][0] - x[i2][0];

dely = x[i1][1] - x[i2][1];

delz = x[i1][2] - x[i2][2];

rsq = delx*delx + dely*dely + delz*delz;

r = sqrt(rsq);

l0 = sqrt(delx0*delx0 + dely0*dely0+ delz0*delz0);

dr = r - 10;

rk = k[type]*dr;
```

4.2. Choices of thermostat algorithms

Ouite a few thermostat algorithms are available in LAMMPS to provide the desired system temperature, such as Langevin thermostat [19], Berendsen thermostat [20] and Nosé-Hoover thermostat [21]. In particular Nosé-Hoover thermostat is one of the most accurate integration methods in molecular dynamics simulations, and LAMMPS users often use Nosé-Hoover thermostat with NVT (constant number of particles, volume and temperature), NVE (constant number of particles, volume and total energy) or NPT (constant number of particles, system pressure and temperature) ensembles to run the simulations of biological system. For running the equilibrium state of vesicle and RBC simulations, we combined the NVT and NPT ensembles to control the system. We observed that using NPT ensemble on water molecules and cytoskeleton network and NVT ensemble for coarse-grained membrane is able to acquire numerical stable configurations. Excerpt from the input script for LAMMPS simulations, we have the following lines to adopt the algorithms:

variable ini_T equal 0.02
variable T equal 0.23
variable P equal 0.05
variable LD equal 1.0
variable P_damp equal 1
fix 1 water npt temp\${T} \${T} \${LD} iso \${P} \${P
\${LD}
fix 2 network npt temp\${int_T} \${T} \${LD} iso \${E
\${P} \${P_damp}
fix 3 bilayer nvt/asphere temp\${T} \${T} \${LD}

4.3. Volume control of water molecule inside the cell

In our CGMD simulations of lipid bilayer membrane dynamics, we have assumed that no internal fluid molecules will be able to move across the membrane or cytoskeleton and vise versa for the solvent. By adjusting the equilibrium cut length of L-J potential for internal water–water interactions, a smaller σ_{eq} implies less neighbors of water molecules to be taken into account, and this means that the water volume inside the cell is reduced. For instance, our goal may be to reduce the vesicle volume during the simulation within a given amount of time. Using the ramp function we can achieve nearly constant rate of change in vesicle volume. In the LAMMPS running script below, we use a ramp function to gradually adjust the equilibrium length σ_{eq} over time to control the cell volume after the desired initial configuration of the cell is obtained.

variable scale1 equal ramp(2.7,2.66)
fix 4 water adapt 1 pair lj/cut sigma 6 6 v_scale1

Fig. 6 is an example, where the green line shows the linear decrease of σ_{eq} from 2.7 at $t \sim 0.8 \,\mu$ s (when the equilibrium configuration is reached) to $\sigma_{eq} \sim 2.1$ at $t \sim 8 \,\mu$ s. The corresponding vesicle volume scaled to the initial vesicle volume v_0 is depicted in the blue curve. We observe that the wiggles of blue curve is due to the thermal fluctuation effect and the trend of decreasing volume is near linear.

4.4. Simulating procedure in LAMMPS

Fig. 7 is the general procedure in which LAMMPS users should follow solid arrows for performing vesicle simulations and both dashed and solid arrows for RBC simulations:

1. For complicated geometries such as RBC which requires an elliptic region for membrane and a hexagonal network for cytoskeleton network, one can use MATLAB script to generate the initial configurations. Otherwise, LAMMPS can handle spherical region or rectangular patch with the command region.



Fig. 6. Dashed line represents the linear decreasing L-J equilibrium length σ_{eq} over time and the solid curve is the volume fraction $v(t)/v_0$ over time. The occurrence of wiggles is due to the effect of thermal fluctuation presented in the simulation. Clearly, the volume of cell is reduced while σ_{eq} is decreasing.



Fig. 7. Flow chart of simulation procedures in LAMMPS. For simple membrane structure such as planar membrane or vesicle, the procedure follows the solid arrows; for RBC simulations all steps connected by dashed arrows are included.

2. Prepare LAMMPS input script to initialize the particles mass, velocities, time step size and the simulation run length.

- 3. Follows from the requirement of LAMMPS data format as shown in Table 1, one needs to call "hybrid" style for using multiple atom styles in LAMMPS. Depends on the subject of simulations, one may need to include the bond styles for specific bonds, for instance, RBC simulations.
- 4. Use proposed pair-potential as the main pair style for bilayer membrane; use L-J potential for membrane-water, cytoskele-ton-water, water-water and membrane-cytoskeleton interactions.
- Setup modified harmonic bond energy for linking proteins between membrane and network. Similarly, setup modified harmonic bond energy for spectrin tetramer and ankyrin bonds.
- 6. For the steps of running equilibrium state, we use the mix of NPT and NVT fixes as our thermostats.
- 7. Adjust the value of σ_{eq} in L-J potential for internal water–water interactions to achieve the cell volume reduction.
- Finally, generate LAMMPS output data into the file format ".lammpstrj".

4.5. Descriptions of the subprograms and sample output

The subprograms include:

- create_rbc_with_water (MATLAB),
- bond_harmonic1 (C++),
- in_example (LAMMPS input script).

We have provided the details of LAMMPS implementations for the LAMMPS input script and modified harmonic bond function in the sections above. Here we include a sample run for RBC simulation where the initial configuration of the RBC is a sphere. The MATLAB script generates an initial data file for the configurations of all atoms including coarse-grained lipid bilayer membrane, cytoskeleton network and water molecules where the generated data file satisfies the file format described above at the beginning of Section 4. LAMMPS is capable to assign specific regions for simple geometries such as planar membrane or spherical surfaces and fill the regions with desired atoms. For this example we require a hexagonal network to represent the cytoskeleton network which is not trivial to be done in the LAMMPS input script. Therefore, with the use of MATLAB script as supplementary tool we can create complicated shapes of objects.

The provided LAMMPS input script dumps a LAMMPS trajectory file (.lammpstrj) and it can be read in various of visualization softwares. For this work Visual Molecular Dynamics (VMD) 1.9.1 is used to generate snapshots from the simulation data and here we show the screen-shot of VMD setting windows and the snapshots of simulation output (without showing water molecules) for this example in Fig. 8.

5. Membrane properties

We first show that the proposed pair potential for coarsegrained lipid molecules reproduces some of the basic membrane properties such as in-plane diffusivity, bending rigidity and membrane tension. For the diffusivity, we study a planar membrane patch where the side $L \sim 40\sigma$ and the particle number *N* is 5822. The time step size is $\Delta t = 0.01\tau$ and we adopt the NVE ensemble with Berendsen pressure control algorithm for 3×10^6 steps. Since the system may take some time to equilibrate, we follow the protocols in the literature [8,9] to average over the last 1.5×10^6 steps for considerations. The diffusivity can be observed by tracking the mean-square-displacement (MSD) which is given by the following formula:

$$MSD(t) = \frac{1}{N} \sum_{i=1}^{N} \langle (\mathbf{r}_i(t) - \mathbf{r}_i(0))^2 \rangle, \qquad (12)$$

where MSD is an accumulated value over the time period *t*. From Einstein's equation for 2D membrane, we can compute the 2D diffusivity by

$$D(t) = \frac{MSD(t)}{4t}.$$
(13)

Fig. 9(a) shows initial state of the membrane patch, and the membrane shape at $t \sim 1 \text{ ms}$ in (b). It is clear that coarse-grained particles move randomly within the membrane as the lipids are in fluid phase. In LAMMPS mean-square-displacement (MSD) can be computed and stored by adding specific commands. Fig. 10 shows MSD and diffusivity as we collect the data every 200 time steps. Fig. 10(a) gives the linearly increasing MSD which represents the pure diffusivity. From the use of Eq. (13), we track the diffusivity over time and the result is shown in Fig. 10(b) which shows the constant diffusion of membrane.

Next we extract the bending rigidity from the membrane fluctuation spectrum. Given a profile function h(x, y) of the planar membrane patch, its Fourier transform

$$\tilde{h}(q) = \frac{1}{L} \sum_{n} h(x, y) e^{i \vec{q} \cdot (x, y)},$$
(14)

where the wave vector $\vec{q} = \frac{2\pi}{L}(n_x, n_y)$ and the wavenumber q is the norm of wave vector \vec{q} . Following [22], the bending rigidity and the membrane tension for a coarse-grained membrane patch in a 3D periodic domain can be approximated by the following formula

$$\langle |\tilde{h}(q)|^2 \rangle = \frac{k_B T}{L^2 (\gamma q^2 + \kappa q^4)},\tag{15}$$

where γ is the membrane tension and κ is the bending rigidity.

For this numerical study, we generate a large membrane patch in a periodic domain with size $L \sim 140\sigma$. The particle number N = 23452 and the time step size $\Delta t = 0.01\tau$. The total steps for simulation is 22×10^6 and we dump the trajectories of membrane into a.xyz file every 1000 time steps. After running 12000τ for equilibrium state, we calculate the values of fluctuation spectra by using 2D Fast Fourier Transform in MATLAB which gives fast evaluations to 2D discrete Fourier transform of height function h(x, y).

Fig. 11 shows that the fluctuation spectra of our simulation results lay on both q^{-2} and q^{-4} lines. From the fitting to Eq. (15), we obtain the approximations of bending rigidity $\kappa \approx 18 k_B T$. This result matches the experimental data of bending rigidity for vesicles and we observe from case studies that the bending rigidity is independent of the size of membrane domain. Therefore, in the following chapter, we perform the numerical applications by using the same parameter set as one used here.

6. Applications to biological systems

We conduct two sets of simulations—vesicle shape transitions and resting shapes of RBCs. Visual Molecular Dynamics (VMD) 1.9.1 is used to generate snapshots from the simulation data.

6.1. Vesicle shape transitions

Yuan et al. used the pair potential in their CGMD simulations of a vesicle and demonstrate the shape transitions of vesicles in [7]. Without using water molecules in their CGMD vesicle simulations, they calculated the volume by local triangulation. The vesicle volume is controlled by a penalty body force from an energy E_V



Fig. 8. Screenshot of VMD setting windows and 3 snapshots from the output of sample RBC simulation. From top to bottom of the snapshots are (1) initial state of RBC; (2) equilibrium state of stress-free RBC; (3) resting shape of RBC after performing the volume control algorithm.



Fig. 9. (a) Initial state of a planar membrane patch where we separate the membrane with two different colors. (b) Membrane configuration at $t \sim 1 \text{ ms} (10^6 \text{ time steps})$. We observe that membrane particles can travel through the membrane behaving as a fluid structure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Fig. 10. (a) Mean-square-displacement (MSD) versus time: linear MSD represents the diffusion property of fluid membrane; (b) From the relationship between the diffusivity and the MSD, we obtain the plot of diffusivity versus time. For this simulation, the parameters are: $\zeta = 4$, $\mu = 3$, $\sin \theta_0 = 0$, T = 0.23 and N = 5822.

that drives the system to a desirable equilibrium volume V from an initial volume V_0 :

$$E_V = \frac{1}{2} K_V \left(\frac{V}{V_0} - 1 \right)^2$$
(16)

where K_V is a constant. In principle we can consider Eq. (16) as a spring energy exerted on the vesicle volume. Therefore, the energy

 E_V should be incorporated into the total free energy. Once E_V is incorporated, enormous amount of computations is needed for local triangulation to the enclosed vesicle membrane at each time step as the volume transitions toward the desirable value [7]. This makes the computation very slow and inefficient.

Rather than local triangulation for volume calculation, we apply coarse-grained particles to explicitly model water molecules with an effective L-J potential for water–water interactions, and adjust



Fig. 11. Blue circles are our simulation results for the fluctuation spectra of membrane height versus the quantities q where q is the norm of two dimensional wave vector. The parameters for this simulation are: $\zeta = 4$, $\mu = 3$, $\sin \theta_0 = 0$, T = 0.23 and N = 23452. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the equilibrium radius σ_{eq} to achieve the volume control. With no penalty energy in the total free energy, at each time step we record the membrane configuration and we are able to compute the vesicle volume by using volume approximation of convex hull in MATLAB.

In the following we report that we can reproduce some of theoretical results using the same set of algorithms in LAMMPS. For vesicle simulations, we only consider a layer of coarse-grained membrane interacting with solvent and calculate the inter-particle force using the proposed pair-potential. We use a spherical vesicle as the initial configuration for of the coarse-grained membrane. The initial vesicle configuration is first generated on MATLAB and a LAMMPS input file is prepared. We note that the file format for hybrid atom styles should comply with the LAMMPS requirement. For example, volume parameter is essential for peridynamics atom style and the volume of each particle is set to be a fixed constant in this simulation.

The parameter set for the pair-potential is the same as in previous work [7]: angular parameter $\zeta = 4$, the parameter $\mu = 3$ is from orientation dependent function $\phi(\hat{\mathbf{r}}_{ii}, \mathbf{n}_i, \mathbf{n}_i)$ in Eq. (4) and θ_0 can be calculated from desired spontaneous curvature c_0 . For the energy unit ϵ to the system we set $k_B T = 0.23\epsilon$ and the cutoff length in pair-potential is $r_c = 2.6$. The thickness of membrane is $\sigma_{ves}~pprox~5$ nm and the diameter of vesicle is $50\sigma_{ves}$. We run simulations in periodic box where the side is $70\sigma_{ves}$. As mentioned in Section 4, we apply NVT ensemble for water interactions and NPT ensemble for the bilayer membrane. We then perform the simulation for vesicle shape transitions by using the ramp function to adjust σ_{eq} in the L-J potential for water–water interactions. Moreover, the equilibrium length of Lennard-Jones potential to the water–water interactions is initially set with $\sigma_{ea} = 2.7$. As described in Section 3, we reduce vesicle volume by decreasing the value of σ_{eq} . From single run simulation with wide range of change of σ_{eq} , say, from 2.7 to 1.5, we are able to track that specific shapes occur when σ_{eq} is at corresponding level. After obtaining the desired vesicle shapes, we unfix the ramp function and relax each case for several time units to obtain the equilibrium states. Finally, we recorded the numerical experiments and corresponding σ_{eq} values. We observed that within certain range of σ_{eq} the vesicle will remain at the specific shapes as shown in Fig. 12.

Our simulation data show that one can obtain prolate shape, dumbbell shape, biconcave shape, stomatocyte-like shape and inward budding cell when $\sigma_{eq} \approx \{2.5, 2.3, 2.0, 1.9, 1.7\}$, respectively. Notice that according to [7], this shape transition can be achieved by using fast rate of volume change \dot{v} within 200 τ where τ is the dimensionless time unit and in real time τ is of order 0.1 µs.

The dimensionless time step size $\Delta t = 0.01$ is used for this simulation and total number of time steps is varying for cases of desired shapes. Fig. 12 shows that starting with spherical vesicle, when the spontaneous curvature sin θ_0 is set to be 0, we have the shape transitions which are prolate (v = 0.8), dumbbell (v = 0.7), biconcave (v = 0.6), stomatocyte (v = 0.65) and inside budding shapes (v = 0.45) with corresponding volume of internal water.

With similar setup, we then perform the simulations for the cases when the spontaneous curvature c_0 is nonzero and the results are shown in Fig. 13. We observed that different from the case when $c_0 = 0$, for $c_0 = 2$ and $c_0 = 4$, the vesicle forms tube like configuration and outward budding shapes.

6.2. Resting shapes of RBCs

Recall the modified harmonic bond function in Section 4, the stress-free configuration of cytoskeleton network plays an important role in the study of resting shapes of RBCs, by minimizing the total elastic energy of the system. Lim et al. compared their simulation results with experiments for resting shapes of RBCs for cases of different spontaneous curvatures and access areas between inner and outer leaflets of the lipid bilayer [12].

In this study, we consider slightly eccentric spheroid as the stress-free configuration of a RBC. Denote the volume of each initial cell as V_s and the volume of original stress-free spheroid cell as V_0 , we performed simulations for the cases when the ratio V_s/V_0 are {0.9, 0.925, 0.95, 0.975, 0.995, 0.998}. To validate our simulations against the continuum simulation results shown in [2], we reduced the volume of RBC from V_0 to $0.65V_0$ and reproduced the results under values of spontaneous curvatures c_0 . Fig. 14 shows the numerical results of stress-free RBC using finite element method (FEM) [2]. With the use of the same parameter set as given in the first part of numerical simulations, one additional NPT fix is needed for cytoskeleton network then we can obtain the stress-free state for both membrane and cytoskeleton.

The experimental observed size of RBC is $6 \sim 8 \mu m$ in diameter but here we inherit the same cell size from previous section where the diameter of cell is $50\sigma_{rbc}$ and the gap between cytoskeleton and membrane is set to be $2\sigma_{rbc}$. As done in the previous simulations, we used periodic boundary condition to run the simulations. With enough amount of coarse-grained particles on membrane and cytoskeleton, the small size of the cell can also achieve the same deformations as ones occurred in real size RBC. In addition, this setup reduces computational cost. We would like to make a remark here that due to the volume of internal water is not fixed for cases of spheroid, σ_{eq} should be carefully adjusted and the cytoskeleton inside the cell takes space therefore the values of σ_{eq} from previous simulation of vesicles are not feasible.

Our simulation results generated from LAMMPS are shown in Fig. 14. Comparing our result with the numerical results from continuum model using FEM, it is clear that for V_s/V_0 is at {0.9, 0.925, 0.95} we have close resting shapes which are biconcave shapes. Also, for V_s/V_0 is at {0.975, 0.995, 0.998} we have bowl shapes which are nearly identical to the results from continuum modeling. We want to point out that the shape transition from bowl shape to biconcave shape is well predicted in this simulation and we include the zoom-in views of both shapes in Fig. 15. Lastly, due to the thermostat generated from LAMMPS, thermal fluctuation would be a huge factor to the simulation in LAMMPS which may cause numerical instability. Thus, the time step size for this simulation is smaller than one in previous sections. Here we used $\Delta t = 0.005$.



Fig. 12. Shape transitions of vesicle for corresponding values of vesicle volume v when the spontaneous curvature $c_0 = 0$. According to [7], this case of transition occurs when the volume change rate is high ($\dot{v} = 1.75 \times 10^{-3} \tau^{-1}$).



Fig. 13. Possible shapes of vesicles when curvature c_0 is nonzero: (a) Tube like configuration ($c_0 = 2$) and (b) outward budding shape ($c_0 = 4$) when v = 0.65, 0.45, respectively.

a V_s/V_0	c_0	0 1	2	3	4	b	V_s/V_0	c_0	0	1	2	3	4	σ_{eq}
0.90							0.90		050	0	C	0	3	2.66
0.925	0		-				0.925		(SR)	S	0	3		2.62
0.95	0	-			\sim		0.95		Ø	9	030	C	(30)	2.60
0.975	0	20					0.975		3	O	0	0	3	2.56
0.995	0	20			\sim		0.995		3	S	0	0	000	2.52
0.998	0	10	20		\sim		0.998		0	0	0	090	3	2.49

Fig. 14. (a) FEM simulation results for resting shapes of RBC reprinted from [2]; (b) Our simulation results using LAMMPS for various spontaneous curvature c_0 versus cases of V_s/V_0 . We also provide the equilibrium length σ_{eq} for internal fluid interactions in each simulation.



Fig. 15. Possible resting shapes of stress-free RBCs: (a) Bowl shape (Stomatocyte); (b) Biconcave shape.

7. Conclusion

In this paper, we incorporate into LAMMPS the pair-potential developed for one-particle-thick CGMD simulations of lipid bilayer membranes. Using LAMMPS we demonstrated that the dynamics of a lipid bilayer membrane immersed in a viscous fluid can be simulated with explicit solvents. We also provide the instruction for preparing the simulation setups consisting of the coarse-grained vesicles, RBCs, internal fluid and solvent. In order to apply the stress-free configuration of the cytoskeleton of RBCs, we modify the built-in harmonic bond energy to acquire the equilibrium state of cytoskeleton network.

Our simulation results show that the orientation-dependent pair-potential for the coarse-grained lipids well captures the membrane properties such as membrane diffusivity, bending rigidity and membrane tension (by evaluating the mean-squaredisplacement and fluctuation spectrum of height function). Furthermore we incorporate coarse-grained water molecules to account for the hydrodynamic interaction between a lipid bilayer membrane and the fluid around it. A Lennard-Jones potential is adopted for interactions between solvent molecules, and by adjusting the equilibrium length σ_{eq} we can control the volume enclosed inside the lipid bilayer membrane or RBC. In our CGMD simulations using LAMMPS we are able to reproduce the shape transitions of vesicle for cases of desired equilibrium volumes. We also perceive the contrast between results in continuum modeling and ones in CGMD for the resting shapes of RBC.

LAMMPS is equipped with the capability for parallel computing with OpenMPI. To illustrate how the approaches presented in this paper may be practical for parallel simulation of realistic biological membranes, here we demonstrate some timing results from parallel computation of the CGMD model of the lipid bilayer membrane: (a) for a total number of CG lipid particles N = 23452running parallel computing on a cluster (with 2.53 GHz 6-core processor) with 160 CPUs, it takes about 3 h to integrate the system to 1 ms; (b) Table 2 shows the timing results of GUV simulations with 144 CPUs. In other words, it becomes practical to couple our pair function with more complicated biological system.

Table 2

Timing results (s) for running 10⁵ time steps of GUV simulations with 144 CPUs.

D _{vesicle}	N _{total}	N _{bilayer}	N _{water}	$T_{100k}(s)$	L _{box}
250 nm	20,011	8,346	11,665	179.70	350 nm
500 nm	147,750	31,404	116,346	439.84	700 nm
1000 nm	1,152,245	125,588	1,026,657	2761.13	1400 nm

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