Graphical user interface, application

Description automatically generatedGraphical user interface

Description automatically generatedGraphical user interface

Description automatically generated with low confidencet

FIG2

FIG4

FIG3

FIG1

Graphical user interface

Description automatically generated with medium confidence

Figure captions:

FIG1:

FIG2:

FIG3:

FIG4:

Methods:

*Some notes to self about interactions in this system.*

*All particles have Volume exclusion via Weeks-Channdler-Anderson potential (Which is a lennard jones with r\_cut= sigma\*2^(1/6). Where sigma is the distance between the centre of one bead to the centre of the other when they are touching (sigma1+sigma2)/2).*

*When we implement an attraction between two types of particles, we extend r\_cut by 1.2 so that the potential extends beyond the repulsive part and we get a short range attraction (as well as volume exclusion)*

﻿**Overview:**

In order to obtain more quantitative results for the dynamics of the system we carried out coarse-grained (CG) molecular dynamics (MD) simulations of an anisotropic particle being wrapped by a membrane. As well as the advantage of the ease of measuring dynamic properties, in these simulations we are also able to control the size of the vesicle, the size of the dumbbell, the membrane tension and the interaction strength between the dumbbell and the membrane. This allowed us to first qualitatively match the simulation system with the experimental set-up and then probe a wider parameter space that is as of yet unavailable in experiments. The membrane is modelled using a 1 particle thick fluid surface developed by Yuan et al [\*\*ref\*\*] which has previously been shown to reproduce the mechanical properties associated with biological membranes. We produce membrane spheres using this model and change the tension of the membrane by producing a pressure force from the addition of small solute particles. Tension is controlled by placing small particles in different concentrations inside and outside the vesicle which only interact via volume exclusion. The dumbbell particle is then placed on the membrane in either a vertical or horizontal initial condition (\*\*\*see fig \*\*\*) and due to the attractive interaction between the membrane beads and the dumbbell, the dumbbell is slowly wrapped and engulfed by the vesicle.

**Simulations details:**

In order to study the effect of osmotic shocks on lipid vesicles we carry out coarse-grained (CG) molecular dynamics (MD) simulations where we explicitly consider the solute modeled as CG particles. The system consists of a spherical membrane surrounded by solute particles on the outside at a concentration r\_out (see purple particles in Fig. 1) and encapsulating inner solute particles at a different concentration r\_in (see cyan particles in Fig. 1). **All particles are volume-excluded spheres of a diameter s, s being the MD unit of length, and mass m =1, embedded in cubic box of constant volume V= L3, with L =44s and within periodic boundary conditions**. See Fig. 1 for the snapshots of the system. **The membrane is modelled using the single particle thick model developed by Yuan et al.34 which is capable of fission and fusion events and reproduces biologically relevant mechanical properties of membranes**. **In this model, bilayer sections of around﻿ 10 nm in width are represented by single beads interacting via an attractive potential. The bending energy is implemented via interactions between vectors on each bead, which represent the local normal of the membrane and are directly related to its curvature**. **The specific parameters of the model are chosen to encode for a fluid membrane of vanishing spontaneous curvature and bending rigidity of 15kBT, kB being the Boltzmann’s constant and T being temperature**. For more details on the interaction potential used in this model and the particular values used here refer to Section VI in the ESI.† All other interactions are modeled as simple volume exclusion repulsive interactions using a Weeks–Chandler–Anderson potential. The system is always initialised in the same way: (i) Nmem =4322 membrane particles define a relaxed spherical vesicle of mean radius R B 17s at the center of the box and (ii) solute particles take random positions on a hexagonal lattice at set concentrations rin and rout inside and outside of the vesicle, respectively, ensuring no overlap occurs with the membrane. In this way, the osmotic conditions of the system are fully defined by two parameters only: the outer solute concentration, rout, and the ratio of concentrations n = rin/rout. The latter quantity is often referred to as the theoretical reduced volume. Hence, setting n o 1 defines hypertonic conditions while n 4 1 corresponds to hypotonic conditions. In this setup the vesicles studied have a diameter of a few hundred nanometers, which is within the range of physiologically relevant scales. However, to test the role of the vesicle size, we also explored larger model vesicles (see Section VII in the ESI†). We run simulations using the open source molecular dynamics package LAMMPS35 with a Langevin thermostat (at reduced temperature T= 1 and damping coefficient of 1) within the NVE ensemble, E being the total ﻿energy of the system, for 3 ? 105 time steps, each of size 0.01 MD time units (t0 ¼pms2e?1 with e = kBT the MD energy unit).

﻿The two beads that engaged in Lennard-Jones (L-J) interactions with the membrane were depicted in darker colors. ﻿The membrane was described with the one-bead-thick model in the fluid state [19]. For simulations of ESCRT-III constriction and cargo sorting, a generic membrane-associated cargo was pre-equilibrated on the membrane layer and placed in the centre of the spirals. ﻿The simulation was performed in the NPH ensemble with the barostat targeted at zero pressure to model a tension-free membrane. The Langevin thermostat was applied at each time step to rescale to the room temperature. Periodic boundary condition was applied to x and y directions. Time step was set as 0.01 τ, where τ is the reduced time unit. All molecular dynamics (MD) simulations were carried out with Lammps code [20].

Outline of Theory: