In self-assembly, the interactions between a collection of components guide them to spontaneously form an ordered structure [1]. Biological self-organization happens within cells, from which all living organisms are composed. Cells are all bounded by a membrane, as are many subcellular structures. Thus many self-assembly processes are membrane influenced. Membranes themselves are also self-assembled, primarily as a lipid bilayer [2]. We focus, however, on structures assembled only from proteinaceous subunits, particularly viruses and clathrin.

The genome of a virus is contained in a core or capsid, a typically monodisperse shell, assembled from individual protein complexes. Often the shells are approximately spherical, with many having icosahedral symmetry [3]. Viruses are divided into enveloped and nonenveloped types, depending on whether the core is surrounded by a membrane. The envelope in the former group is acquired through budding [4]. For both enveloped [5–10] and nonenveloped [11–13] viruses there is abundant evidence of membrane influence on core assembly. Clathrin, on the other hand, is intrinsically linked to membranes: its main function is the formation of coated vesicles for intracellular protein transport [14]. Its three-legged shape allows a collection of individual units to form structures that range from extended hexagonal sheets to closed cages, whose highest end gives membranes that are easily deformed, and lowest end gives ones that may not be deformed, and whose highest end gives ones that may not be deformed, by the assembled structures. Here we focus on equilibrium, postponing dynamics to a later work.

Our model comprises $N_{SU}$ assembling subunits plus the membrane. The former are modeled as spherical...
patchy-particles with a Kern-Frenkel potential [21], similarly to previous work [25], but modified suitably such that its first derivative is continuous. The membrane is represented using a dynamically triangulated surface model [36]: \( N_{\text{mem}} \) particles connected with \( 3N_{\text{mem}} \) bonds form a network of \( N_{\text{mem}} = 2N_{\text{mem}} \) triangles. We sample using Monte Carlo (MC) simulations [37], performed in a periodic rectangular box of sides \( L_x, L_y, \) and \( L_z \). The membrane’s projection completely covers the box in the \( xy \) plane, connecting to itself across the boundaries. To apply no external tension [38] we allow \( L_x = L_y \) to vary, while also adjusting \( L_z \) to keep to volume \( V = L_x L_y L_z \) fixed. The MC moves used do not allow the membrane topology to change. Subunits interact with the membrane from both sides but are only attracted to one side. Quantities are given in units of the thermal energy \( k_B T \) or the typical length of a membrane bond \( l \).

Interactions between subunits (ss) and between subunits and membrane particles (ms) are of a Lennard-Jones type (see Supplemental Material Ref. [39]). An orientational dependence of the attractive part creates patches. The parameter \( \theta_0 \) defines the maximum angular deviation of the patch position from the particle-to-particle vector before the attractive interaction decreases. It is chosen such that, for a given pair of subunits, only one pair of ss patches can interact at once. For ss interactions, twisting of subunits around an interacting patch is also penalized, mimicking the torsional constraints in protein-protein interactions [25]. The ss patches are evenly spaced around the particle, with the ms patch lying on the axis of rotational symmetry. The subunits have a size of \( \sigma_s = 2.5 \) and we choose \( \theta_0 = \pi/4 \) for the ms interactions. This gives a relatively wide ms patch, so that a subunit typically interacts with many membrane particles so seeing a smooth surface. The minima of the ss and ms interactions are \( -\epsilon_{ss} \) and \( -\epsilon_{ms} \). A pair of subunits are defined to be bonded if their interaction energy is \( < -0.25\epsilon_{ss} \). The bending stiffness of the membrane is set by \( \lambda_b \approx \kappa \), the bending rigidity of the membrane (see Refs. [36,39]).

We choose two different parameter sets. For our core model, \( N_{SU} = 12 \). Subunits have five ss patches with \( \theta_0 = 0.2 \), giving a similar patch width to the optimum in Ref. [25]. If the twelve subunits are placed on the vertices of an icosahedron, they may be aligned with the ms patches pointing outwards and every ss patch pointing directly at a patch on a neighboring subunit, bonding with it. Here, \( V = 1.07 \times 10^4 \) and \( N_{\text{mem}} = 576 \). In our clathrinlike model \( N_{SU} = 36 \) and subunits have three ss patches each with \( \theta_0 = 0.3 \). The patches are wider to allow for a range of curvatures. Following Ref. [28], the ss patches make an angle of \( (79/180)\pi \) to the ms patch so that, if a closed cage is formed, the ms patches point inwards. Here, \( V = 2.15 \times 10^4 \) and \( N_{\text{mem}} = 1156 \). Membrane sizes were chosen, using preliminary runs, to give plenty of area to cover assembled structures, with the \( V \) chosen to allow large membrane deformations. Qualitative results were not sensitive to \( V \).

The main connection to biological systems is that the interactions drive our models to form similar structures. The core subunits resemble intermediate capsomers in the assembly of a \( T = 1 \) capsid, the smallest virus structure with icosahedral symmetry [25]. In reality, enveloped viruses are larger. In our clathrinlike model subunits are considered equivalent to one clathrin, with each patch representing a leg. This is a simplification in that, in structure formation, multiple legs of different clathrin lie along each other. We neglect adaptor proteins [14].

Efficient sampling of our system must overcome a number of issues: free-energy barriers between assembled and disassembled states; the importance of collective motion for membrane relaxation; large times to find target structures. MC simulations allow us to combine different approaches that address these problems, specifically aggregate volume bias moves [40], hybrid Monte Carlo (HMC) moves [41], and multicanonical parallel-tempering [42]. The aggregate volume bias moves, which shift subunits directly between nonbonded and bonded states, as well as displacing bonded clusters onto or off the membrane, allow target structures to be found very quickly. HMC, which uses molecular dynamics trajectories to create trial states, captures collective motion. Finally, the free-energy barrier problem is ameliorated through the use of multicanonical parallel-tempering, involving parallel tempering swaps in two dimensions, \( \epsilon_{ss} \) and \( \epsilon_{ms} \). The further addition of a one-dimensional biasing potential, \( w(E_{ss}/\epsilon_{ss}) \), constructed iteratively during initialization, increases the swap acceptance rate. \( E_{ss} \) is the total interaction energy between all subunits. We found that the HMC
We define a core to be assembled if all twelve subunits are in a cluster and each makes five bonds. In Fig. 1 we plot the probability of finding an assembled core, \( P_a \), as a function of \( \epsilon_{ss} \) and \( \epsilon_{ms} \) for a range of \( \lambda_b \) between \( \sqrt{3}/2 \) and \( 8/3 \). Our chosen \( \epsilon_{ss} \) range covers the crossover from \( P_a = 0 \) to \( P_a = 1 \) for a bulk system with the same free assembly volume. For all \( \lambda_b \), we observe that, for the lowest \( \epsilon_{ms} \), this crossover occurs at about the same \( \epsilon_{ss} \) as in the no-membrane system (see Supplemental Material Ref. [39]).

For more deformable membranes, as \( \epsilon_{ms} \) is increased, assembly occurs at lower \( \epsilon_{ss} \). This enhancement depends nonmonotonically on \( \lambda_b \), see Fig. 1(d), occurring over a larger area of parameter space for \( \lambda_b = \sqrt{3} \) than for \( \lambda_b = \sqrt{3}/2 \), but then reducing and disappearing as \( \lambda_b \) is increased further. For lower \( \lambda_b \) and high \( \epsilon_{ms} \), the membrane tends to envelop the subunits. In Fig. 2, typical configurations observed for \( \lambda_b = \sqrt{3}/2 \) and \( \lambda_b = \sqrt{3} \) with an assembled core attached to the membrane are shown. Interestingly, while for \( \lambda_b = \sqrt{3} \) this envelopment is almost complete, forming a bud, for \( \lambda_b = \sqrt{3}/2 \) it is only partial. In Figs. 2(c) and 2(d) we plot the average of the total membrane-subunit interaction energy, \( \langle E_{ms} \rangle \), as a function of \( \epsilon_{ss} \) and \( \epsilon_{ms} \) for the same \( \lambda_b \), confirming that for \( \lambda_b = \sqrt{3}/2 \) the membrane envelops the subunits less: for \( \lambda_b = \sqrt{3} \) the minimum of \( \langle E_{ms} \rangle \) is \( \approx -100 \), while for \( \lambda_b = \sqrt{3}/2 \) it is \( \approx -70 \). The lowest \( \langle E_{ms} \rangle \) are strongly correlated with envelopment in buds.

For \( \lambda_b = 2\sqrt{3} \), some configurations with similar envelopment as for \( \lambda_b = \sqrt{3} \) are seen but, for higher \( \lambda_b \), only some deformation, not full envelopment, is seen (see Supplemental Material Ref. [39]). The lack of an enhancement of assembly in this regime, despite strong attractions to the membrane, is in contrast to the case of extended crystals, where structures grow near attractive walls even if the bulk is fluid [44].

In the bulk, the probability of assembly is determined by whether the attractions are sufficient to overcome the associated entropy loss. The attraction of subunits to the membrane confines them, reducing this entropy loss, which may promote assembly. If the free energy gain in forming the core is sufficient to overcome the bending energy, as well as the entropic cost of binding to the core, the assembled structure may form a bud. Budding is not necessary for assembly promotion but the membrane stiffness with the most budding also has the most promotion. The nonmonotonic rigidity dependence may be due to budding suppression: for low stiffness by membrane entropy and for high stiffness by bending energy. For our cores, changing the membrane stiffness and attraction changes the probability of forming one specific structure. For isotropic particles, in contrast, altering these parameters may lead to qualitatively different structures [30,31].

While our range of bending rigidities approximately overlaps with that expected for biological membranes (= 2.5–25 [45]), those where we see budding are somewhat on the lower side of this range (\( \lambda_b \leq 2\sqrt{3} \)). This discrepancy may well arise from the coarse-grained nature of our model and particularly from the relatively small number of subunits in our cores: assuming the free energy gain is proportional to the number of subunits forming them, smaller structures will be less able to deform the membrane into a given shape.

For the clathrin-like model, the structures formed are typically polydisperse, see Fig. 3, and we use a standard measure of asphericity, \( \Delta \), to investigate their shape. \( \Delta \) (see Refs. [39,46]) takes values between 0 and 1, with 1 corresponding to a shape with spherical symmetry and 0 corresponding to a nonspherical, oblate, or prolate shape.

We first focus on the average of the number of subunits in the largest bonded cluster, \( \langle N_{max} \rangle \). We consider the same range of \( \epsilon_{ms} \) as for the core model and choose the \( \epsilon_{ss} \) range so that for the no-membrane system it covers the crossover from small clusters of a few subunits to most of the 36 subunits being in one cluster (see Supplemental Material Ref. [39]). For higher \( \epsilon_{ss} \), without a membrane, the subunits are observed to form closed cages; see Fig. 3(a). We observe that the subunit bonds form 5 and 6 member closed
enhancement remains as roughly spherical structures which enclose membrane
Fig. 2. However, as shown in Fig. 4(b), in contrast to cores, the intermediate
where $\lambda_b = 8 \sqrt{3}$. Color and gray tones as in Fig. 2.

![Fig. 3](color online). Typical configuration from simulations with the clathrinlike model: (a) $\epsilon_{ss} = 12$, without membrane. (b) $\epsilon_{ss} = 12$, $\epsilon_{ms} = 1$, $\lambda_b = \sqrt{3}/2$ (c) $\epsilon_{ss} = 12$, $\epsilon_{ms} = 1$, $\lambda_b = 2 \sqrt{3}$. (d) $\epsilon_{ss} = 12$, $\epsilon_{ms} = 1$, $\lambda_b = 8 \sqrt{3}$. Color and gray tones as in Fig. 2.

Fig. 3 (color online). Typical configuration from simulations with the clathrinlike model: (a) $\epsilon_{ss} = 12$, without membrane. (b) $\epsilon_{ss} = 12$, $\epsilon_{ms} = 1$, $\lambda_b = \sqrt{3}/2$ (c) $\epsilon_{ss} = 12$, $\epsilon_{ms} = 1$, $\lambda_b = 2 \sqrt{3}$. (d) $\epsilon_{ss} = 12$, $\epsilon_{ms} = 1$, $\lambda_b = 8 \sqrt{3}$. Color and gray tones as in Fig. 2.

tens on the cage surfaces but the shape of the faces they enclose deviate significantly from pentagons or hexagons, being not generally flat, and the cages, while qualitatively similar, are not generally of the form of the structures observed for clathrin [15]. The key difference may be that when two clathrin bond their legs lie along each other, which will result in a greater flexibility to tilt the symmetry axes of the two clathrin than to rotate around the symmetry axes. In our clathrinlike model, however, a bond has equal flexibility for both such deformations.

In Figs. 4(a) and 4(b) we plot $\langle N_{\text{max}} \rangle$ as a function of $\epsilon_{ss}$ and $\epsilon_{ms}$ for $\lambda_b = \sqrt{3}/2$ and $8 \sqrt{3}$. Looking at Fig. 4(a) we see that, for the most flexible membrane, there is a similar enhancement of assembly for higher $\epsilon_{ms}$ as for cores. However, as shown in Fig. 4(b), in contrast to cores, the enhancement remains as $\lambda_b$ is increased. The results for all intermediate $\lambda_b$ were very similar (see Supplemental Material Ref. [39]). This is due to the ability of the clathrinlike subunits to form structures with a range of curvatures; as depicted in Fig. 3(b), at lower $\lambda_b$ the subunits form roughly spherical structures which enclose membrane buds. Indeed, at $\lambda_b = \sqrt{3}/2$, these are often nearly closed, with the membrane in the bud connected to the rest by a very narrow neck (see Supplemental Material Ref. [39]). At intermediate $\lambda_b$, we observe the formation of more open, curved structures, or pits, on the membrane, see Fig. 3(c), whereas at high $\lambda_b$ the subunits form extended, approximately flat structures lying on the membrane surface, as shown in Fig. 3(d).

In Fig. 4(c) and 4(d) we plot the average of the asphericity of the largest subunit cluster, $\langle \Delta \rangle$, for $\lambda_b = 2 \sqrt{3}$ and $8 \sqrt{3}$. For low $\epsilon_{ms}$ and higher $\epsilon_{ss}$ there is a region for all $\lambda_b$ where $\langle \Delta \rangle = 0$, corresponding to closed cage structures, not attached to the membrane. For higher $\epsilon_{ms}$, nonclosed structures bound to the membrane are formed. At lower $\lambda_b$ these remain somewhat spherical, while for $\lambda_b = 8 \sqrt{3}$ they are a lot less so.

To summarize, we described a simple coarse-grained model to simulate the effect of a membrane on the assembly of proteinaceous subunits. We used this model to investigate the assembly of structures that share key features with viral cores and clathrin. In both cases we found that attraction to the membrane may enhance assembly in regions without bulk assembly. For cores, this effect shows an interesting nonmonotonic dependence on membrane rigidity, being reduced for very deformable membranes and disappearing for the stiffest, in contrast to extended crystalline structures with attractive walls. For clathrinlike particles, the promotion of assembly persists for less deformable membranes. The difference to cores is due to the ability of clathrinlike particles to form structures with different curvatures. Furthermore, we observed the formation of biologically relevant buds for both cores and clathrinlike particles. In the case of cores, we found that these do not occur if the membrane is very flexible, while for clathrinlike particles their morphology depends on membrane flexibility.

The formation of buds on membranes is crucial in various biological processes, for example endocytosis, in which, in some organisms, clathrin plays an important role. Endocytosis is a complex process involving the collaborative binding of a variety of proteins to the membrane [47]. The demonstration of bud formation through assembly in our simulations opens the possibility that simple, patchy-particle models could capture basic features of such processes, giving new insight. The effects described might
also be experimentally observed by mixing patchy colloids [48,49] with giant vesicles [50,51], whose bending rigidity [52] lies well within the range considered. More generally, our results clearly demonstrate that membranes can have a profound impact on self-assembly and will hopefully stimulate further study in this direction. In the future it will be interesting to investigate the dynamics of membrane-influenced assembly.

This work was supported by the Austrian Science Fund (FWF): Grant No. M1367. Snapshots were created using VMD [53]. The computational results presented have been achieved in part using the Vienna Scientific Cluster (VSC).