**Review of Curvature Models**
**and Up-Down Symmetry Broken Shapes**

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for MIT 8.592

**Abstract/Introduction**
We review selected aspects of two-dimensional flexible surfaces with a particular focus on axial symmetric shapes with broken up-down symmetry, such as pears and cones. Natural examples of such shapes include HIV viral capsids, cell membrane buds, and red blood cell spicules. By establishing the spatial extent of individual cells and viruses, closed 2-d vesicles play an existential role in living systems. The great variety of living shapes arises from flexing modes of these surfaces interacting with cytoskeletal elements in the non-equilibrium chemical environment of the cell.

First, we discuss continuum models of bilayer vesicles, which have succeeded in describing red blood cells and budding processes. A discussion of micellar phases formed by the bilayer's molecular components leads to a discussion of viral capsids, in particular the unexplained shape of the HIV virus. We conclude with a brief discussion of open questions.

**Vesicle Bending Models**
Three centuries after Robert Hooke coined the term "cell" (1664) and wrote his linear theory of elasticity (1678), Canham (1970) and others applied this theory to understanding the shapes of mature red blood cells (RBCs). Experiments by Rand (1964) and others had shown that RBCs could withstand large shape deformations and return to their resting shape. Further, a variety of environmental factors such as pH, proximity to a glass plate, ionic solution strength, and others, can change the resting shape. To explain the highly repeatable sequence of resting shapes (Figure 6), earlier workers ignored the bending energy of the membrane and focused attention on stretching energy and other factors. Their own experimental work suggested that the membrane's bending energy can be as large as its elastic energy, at least when an RBC assumes the spikey echinocyte shape (Canham 1970).

Realizing the importance of the bending term, Canham numerically solved the bending term alone and found minimum energy shapes that closely matched RBC. This watershed event established a corner stone of current vesicle theories. See Figures 1 and 2 for excerpts from Canham's original paper.

The now-standard derivation of this bending term starts with deformations written in terms of a coordinate system defined on the membrane. The fluid nature of the membrane requires that all orientations of the coordinates should yield the same answer, so one generalizes to invariants of the 2-d surface: the mean and Gaussian curvature. The Gauss-Bonnet integral is typically ignored as a constant contribution to the energy.

Since the inner and outer membrane layers experience different chemical environments, one might expect small patches of the surface to prefer to bow inward or outward. Helfrich extended Canham's harmonic bending term to describe membranes whose unconstrained resting shape is non-planar. This "spontaneous curvature" (SC) breaks symmetry by preferring shapes with curvature closer to $C_0$. The Canham-Helfrich model is invariant under rotation of the reference vectors tangent to the surface, so it preserves symmetry within the tangent plane as required by the fluid nature of the amphiphiles in the membrane.

The two lipid monolayers that form the membrane bilayer appear to not interact appreciably. They can slide past each other with little observable effect. However, if the membranes are geometrically constrained to have the same boundary conditions, such as in a vesicle, then the two sides must move together. If such a constrained bilayer is curved, the two sides stretch different amounts (see Figure 3). Conversely, if the chemical environments of the two sides differ, they may contract or expand differentially and thus cause the membrane to curve. Based on geometric analysis of this difference, Evans (1974) introduced the non-local term shown below. This "bilayer-coupled" or "area difference elasticity" (ADE) plays a crucial role in describing budding and RBCs.

To leading order in the membrane thickness, the SC and ADE models differ only by the placement of a square. Expanding the two squares makes the non-locality obvious. $\Delta A$ is the difference in area between the two bilayers. H is the standard mean curvature, which is half the trace of the curvature tensor. K is the Gaussian curvature, which is the determinant of the curvature tensor.

$$E_{SC} = \frac{\kappa_b}{2}\oint (2H - C_0)^2 dA \ldots\ldots\ldots\ldots \propto \oint (H - H_0)^2 = \oint H^2 - 2H_0 \oint H + {H_0}^2 A$$

$$E_{ADE} = \frac{\overline{\kappa}}{2}\frac{\pi}{AD^2}(\Delta A - \Delta A_0)^2 dA \propto \frac{1}{A}(\oint H - H_0)^2 = \frac{1}{A}(\oint H)^2 - 2H_0 \oint H + {H_0}^2 A$$

$$\Delta A = D\oint H dA + O(d^2)$$

Including Canham's initial work, several groups have performed numerical searches for minimum energy shapes of these models. Seifert et al (1991) completed low-curvature phase diagrams for axisymetric shapes for the spontaneous curvature model and the area difference model. The surface areas and volumes of living cells tend to remain constant over time periods of hours, so the shapes are characterized by the reduced volume (Figure 4), which ranges from zero for densely crumpled membranes to one for a perfect sphere. These solutions are "low-curvature" in the sense that the characteristic radii of the area, volume, and preferred curvature are all of the same order of magnitude. High curvature shapes have a preferred curvature radius much smaller than the area or volume radius.

The non-local nature of the area difference term creates long-range interactions. This is essentially mean-field in character. Thus, it is not shocking that for ADE, Seifert finds, "all shape transformations are continuous, i.e. the first derivative of the energy with respect to v and $\Delta a$ is continuous at the transformation point (Seifert 1991)." The spontaneous curvature term yields mostly discontinuous transitions. See Figure 4 for examples of stable pear shapes that at different values of the area difference.

Small deformations from the sphere occur along different $Y_{lm}$ branches. Starting with a general expansion of the energy, Peterson (1989) showed that all l¾3 shapes are unstable to l=2 (elliptic) perturbations. For this reason, Seifert explored only shapes arising from the l=2 branches, which are prolate and oblate ellipsoids, see Figure 5 (reproduced from Seifert's phase diagrams). Both diagrams have gaps between oblate and prolate shapes. In section IVC, Seifert points out that if any stable shapes exist in this gap, they must be non-axisymmetric. This means that non-axisymmetric solutions might exist for all values of the reduced volume except $v$=1. Wintz (1996) showed that especially for small values of $v$, a great variety of starfish-shaped vesicles have nearly the same energy. These collapsed vesicles resemble the zoology of micelle shapes discussed by Bouligand (below). While a complete enumeration of such shapes is not practical, one can explore them easily using Surface Evolver (Brakke 1992).

Using Surface Evolver, one can quickly generate many of the shapes that Seifert enumerated exhaustively. Seifert numerically integrated the five ODEs describing volume- and area-constrained axisymmetric shapes to find all the edges in the phase diagram.

Seifert found pear-shaped and stomatocyte solutions, which break the up-down symmetry present in small deviations from a sphere. Budding did not occur in the low curvature part of the diagram. By exploring the high-curvature phase diagram of both the SC and ADE models, Miao (1991, 1994) found budding transitions in which a large vesicle sheds small vesicles of the preferred radius. This budding process is quasistatic in the sense that it is always in a set of equilibrium shape. Using the Surface Evolver, one can observe budding *in virtuo* by setting a large spontaneous curvature and iteratively increasing the target area and evolving to a minimum energy shape. As discussed in Miao, the numerics can be quite sensitive as the necks pinch off. Through this process, large vesicles reduce their energy by shedding chains of small vesicles.

Factors such as pH changes that cause fluid vesicles to bud do not cause RBCs to bud. Instead, the RBC forms spicules that do not detach. The RBC cytoskeleton holds the membrane together and prevents neck cleavage. The 140 $\mu m^2$ membrane area of a typical RBC is decorated with about 120,000 spectrin filaments, with an average separation of about 75nm. The spectrin are attached near the midpoint of their lengths with their ends connected by junctions made of actin. Typical spectrin filaments are 200nm long and have a persistence length ten times smaller. Thus, unstretched spectrin can assume a great variety of shapes and act as entropic springs. After a forced deformation, these springs pull the membrane back to the average separation of 75nm. (Boal 2002, pg. 45, 59).

Lim (2002) reproduced the continuous transformations between resting shapes of the RBC using a Canham-Helfrich-Evans model combined with an elastic and shear energy for the RBCs spectrin cytoskeleton. Lim included elastic and shear terms up to fourth order to account for the sharp corners in the spiculated shapes (Figure 5 and 6). To reproduce the shape sequence observed in real RBCs, Lim varied the preferred curvature parameter (see Figure 5). This lends further support to Evans' non-local ADE model. The model's success on budding and RBCs establishes the current foundation for theories of membrane-cytoskeleton interactions.

Starting with a general expansion of the energy, and keeping all terms up to second order, Helfrich argued that only three elastic modulii are independent for fluid vesicles: a surface tension (usually neglected), a bending rigidity ( $\kappa$ ), and a Gaussian rigidity ( $\bar{\kappa}$ ) (see David's chapter in Nelson 2004, and Miao 2002). When asymmetries of the molecules making up the membrane cause the second order rigidities to be zero, one must consider higher order terms. With such asymmetries as motivation, Mitov (1978) presented higher order terms including powers and local derivatives of $H$ and $K$.

Mitov's terse presentation in an obscure journal has not attracted much attention. In fact, a couple incomplete re-attempts have occurred. Fogden (1991) attempted to model such asymmetric amphiphiles by inserting two different spontaneous curvatures into the second order terms. By forming only a few combinations ($c_1 - \gamma_1$) and ($c_2 - \gamma_2$), this misses many terms permitted by the symmetries. Bouligand (1999) takes a similar approach, but his geometrical development utilizing parallel planes through the thickness of the bilayer appears likely to generate more of Mitov's terms. Bouligand stops short of writing them down.

Phase diagrams resulting from such higher order terms do not appear to have been worked out.

**Micelles**

Vesicle membranes are aggregations of amphiphilic molecules, which have a water soluble head region and an insoluble hydrocarbon chain (Boal, Appendix B). In low concentrations the amphiphiles form surfactant films that behave like 2-d gases on the surfaces of water. In higher concentrations, amphiphiles form micelles in a zoology of arrangements that protect the tails from water contact. Gompper and Schwartz (1995) found that a Landau-Ginzberg energy functional with a vector and a scalar order parameter can reproduce the experimentally observed phase diagram of micelles in binary mixtures (water and amphiphile) and ternary mixtures (water, oil, and amphiphile). The scalar order parameter represents the concentration of the amphiphiles, and the vector field represents their local orientation.

As can be seen in the many exotic varieties of micelles (cf Bouligand 1999), open edges do not occur in equilibrium configurations. Edge energies are eliminated when the sheets close on themselves and form 2-d surfaces. *In vivo* observations involve surfaces isomorphic to spheres almost exclusively. However, wet-lab and numerical experiments have found toroids, higher genus shapes, and periodic minimal surfaces (Gompper 1995, Brakke's Surface Evolver web site.)

Amphiphiles form several stacked phases where individual bilayers stack in parallel. In the $L_b$ stacked phase, the

amphiphiles are hexagonally close-packed in the plane while they remain free along the normal direction. This liquid crystal phase possesses long-range order (Nelson and Pelliti 1987). Similar p-atic ordering occurs in polymerized membranes (see review by Gompper 1997). A primary result from this analysis is that conical buckling occurs around defects. If the lattice is held flat, the defect costs energy proportional to the area of the lattice. This diverges with system size. Such long-range interactions can be visualized by cutting a wedge out of a paper plate, and trying to reconnect the two edges without allowing the paper to leave the plane. By allowing buckling out of the plane, the paper plate can obviously form a cone. This costs a much lower energy, which is logarithmic in the system size (Nelson and Pelliti 1987).

The interaction between such defects is an active area of research. Pentagons have positive topological charge. When a hexagonal lattice is draped over a bump, any defects in the lattice "feel" the bump. The minimum energy configuration occurs when the positive charge defect is placed at the top of the bump. If one imagines that the lattice can slide over the bump to minimize its energy, then one can say that the pentagon moves to the top of the bump. Seven-fold cells have negative charge and move to infinity. For a Gaussian-shaped bump, dipole-like pairs of 5 and 7-fold defects move to the edge of the bump with the positive cell closer to the top of the bump. Such dipole-like defects are called dislocations. Single-cell defects are called disclinations. Multiple pentagons allowed to move independently in a hexagonal lattice will repel each other. However in the presence of a bump, they will arrange themselves symmetrically around the sides of the bump, thereby balancing their mutual repulsion and their attraction to the top of the bump (Lucks not yet published, Vitelli 2004a, 2004b, and personal communication).

Defects like these occur in p-atic surface models that consider a vector order parameter trying to align with its neighbors on the surface. As is pictured in Vitelli 2004b, a lattice draped over a taller bump is afforded a longer waist region in which to align such an order parameter. Such elongated cylindrical necks that do not pinch off are thus intuitively expected in vesicle models with such an energy term.

Such buckling around defects is crucial to understanding viral capsids, which form from polymerized amphiphiles. Viral capsids are composed of single proteins that have head groups that fit together to form hexamers and pentamers. The tail groups of these proteins are generally hydrophobic, and in solution, they dimerize. The dimers thus form the bonds of a hexagonal lattice.

**HIV's Conical Shape**

The relevance of such hexagonal ordering was partially anticipated by Watson and Crick (1956) when they hypothesized that viruses must form Platonic solids. Platonic solids can be constructed from identical subunits. Since viruses only have room for a few proteins in their genomes, Watson and Crick deduced that most viruses would code for only one capsid protein and must therefore form Platonic solids. Indeed, most viral capsids have the symmetry of the largest Platonic solid, the icosahedron (60 sides). Shapes with more than 60 elements can have icosahedral symmetry when the twelve pentagonal "defects" of the icosahedron are spaced farther apart.

Caspar and Klug (1962) classified such icosahedral shapes by counting the number of hexagons that separate the pentagons in these sphere-like objects with icosahedral symmetry. Any sphere-like object with icosahedral symmetry is characterized by its T-number, $T=h^2 + k^2 + h\,k$ , where h and k are integers describing the number of lattice sites separating the twelve pentagons. As the T number increases, the sphere gets larger and the angle between adjacent hexamers decreases. This edge angle is constant through the sphere and must be spontaneously preferred by the capsomers in order for the capsid to self-assemble. Thus, this spontaneous curvature is the natural thermodynamic control parameter for capsid size (Caspar and Klug 1962, cf. Bruinsma 2003). This took place a decade before Canham, Helfrich, and Evans' work.

The comparatively well understood self-assembly of micelles provides a spring board for hypothesizing how viral capsids form. The small size of viruses suggests that higher curvature terms may be needed, and the polymerized lattice of capsomers suggests a vector order parameter on the surface.

Not all viruses are spherical. HIV forms cone-shaped capsids (Ganser 1999, Li 2000). This up-down symmetry broken shape requires the capsomers to change out-of-plane bond angle through out the length of the cone.

After replicating its RNA in a host cell, HIV spreads to other cells by forming a conical viral capsid surrounded by a membrane vesicle that buds off the host cell. The HIV's RNA genome codes for the Gag polyprotein, which is cleaved into three standalone proteins: "the NH2-terminal MA (matrix) region binds the membrane, the central CA (capsid) region mediates important Gag-Gag interactions, and the COOH-terminal NC (nucleocapsid) region packages [condenses around] the viral RNA genome… [The] RNA/NC copolymer is surrounded by an outer shell composed of ~1500 copies of CA. The conical core appears to be essential, because Gag mutations that disrupt proper core formation invariably inhibit viral infectivity." (Ganser 1999) Ganser et al observed that NC and CA can form cones and cylinders *in vitro*. In high ionic strength solutions (1 M NaCl) these shapes emerge even without scaffolding help from the virus' RNA genome. With non-specific scaffolding, cones and cylinders form in a ~2:3 ratio (Ganser 2000). *In vivo* ratios of cones to cylinders are ~20:1 (Li 2000).

As illustrated in (Figure 7), hexagonal lattices can form five seamless fullerene cones with angles precisely determined by arranging 1, 2, 3, 4, or 5 pentagons at the apex of the cone. Even without an assembled apex, Euler's theorem guarantees only five permissible cone angles. To see this, look at the cuts of the plane in Figure 7. Cone angles observed in HIV CA assemblies match these five angles. The cones occur with different frequencies: the narrowest cone (P=5) is far more

common (see Figure 8.) For cones of the same length, larger cone angles would expose a larger perimeter of dangling hydrophobic tails with a correspondingly larger energy cost. This might account for the different frequencies (Zandi personal comm.)

Ganser et al borrowed the fullerene cone model from studies of carbon structures by Ge (1994) and Krishnan (1997). Ge observed exclusively P=5 cones (apex = 19.2°). To explain this, Ge points out that the carbon cones grow from seed groups. He suggests that groups of 2, 3, and 4 pentagons would cause higher strain in the transition region than the nearly hemispherical P=5 cap. He suggested that while P=1 cones would have acceptably low strain, they would be close to flat and might elude observation.

Krishnan (1997) observed cones of all angles and contradicts Ge by stating unambiguously that energy arguments would lead one to expect smaller numbers of pentagons, because they have smaller strain. A number of competing models have been proposed for fullerene assembly. Since Krishnan observed cones of all angles with a broad peak around P=3, Krishnan concludes that strain energy cannot be the driving factor. Instead, he proposes that the assembly process prefers intermediate steps that increase the entropy of the configuration. Carbon tends to form rings of 10 to 30 units. These rings may form the building blocks of fullerenes. As an example, he says that a ring of 16 carbon atoms can arrange around a P=2 apex in 2,500 different configurations compared to 16 configurations when arranged around a point in a flat lattice. Such entropic considerations might also effect HIV formation.

Ge points out that the five fullerene cones have differing symmetry. While all five possess a mirror plane symmetry, the odd numbered cones (P=1,3,5) have an armchair and a zigzag lattice pattern at the lines where the mirror plane crosses the cone, and the even numbered cones have zigzag lattices on both lines. This geometry can also be seen from drawing caps for the apex of the cone with the required number of pentagons. These boundary conditions on the each type of cone match with the corresponding cap when the pentagons are arranged with T=1. Perhaps this is required for carbon, however for HIV cones, it appears that higher T-number arrangements of the apex cap are geometrically possible and might cap the cylinder at arbitrary radii.

The fullerene cone structure has constant in-plane bond angle and requires changing out-of-plane bond angle throughout the length of the cone. To understand what microscopic properties of the HIV CA protein allow this, Li (2000) reconstructed detailed images of the proteins occurring in cylinders using electron cryo-microscopy. The CA protein consists of two subunits, the N-terminal domain and the C-terminal domain, connected by a linker. They found six different helicities in the CA tubes, which suggests that the linker section between the two domains exhibits "unprecedented" flexibility. The packing distance between the hexamers can vary and the angles between neighboring hexamers "can adopt essentially any orientation with respect to the helix axis." This flexibility could allow capsid volume to vary, which Li suggests would allow the virus to incorporate genes from the host organism. Regular icosahedral viruses might not be able to fit a larger genome without evolving a new capsid protein.

**Discussion**

If a volume constraint is required to break up-down symmetry, what might constrain the volume of the HIV capsid – the scaffolding provided by the genome? If the HIV CA linker is so flexible, why does it form the cone at all? Is it a trapped kinetic state? What accounts for the 2:3 and 20:1 difference between cones:cylinders *in vitro* versus *in vivo*?

One might ask what general properties can cause a simple energy model to have minimum shapes with broken up-down symmetry. The shapes considered by Seifert, Miao, and others are constrained by volume and surface area. Shapes built from spheres, cylinders, and cones can be solved analytically to gain insight (see Boal 2002 pg 219 for a table). Using such "limit shapes" with few parameters, one can find minimum energy shapes by hand. For example, in Figure 9 we show that for two spheres without a constraint on area or volume, the spontaneous curvature model cannot break up-down symmetry. Only a volume constraint can force the minimum energy shape to have one side bigger than the other. A similar analysis for a cylinder deforming into a frustum gives the same result. Can an area constraint alone ever yield shapes with broken up-down symmetry?

Analyzing combinations of shapes of constant curvature is facilitated by judicious use of Buckingham's Pi theorem. For example, by choosing the mean radius to be one, a series of cylinders with radii arranged around the mean radius leads to an analytically tractable summation over the interpolation function (see Figure 10). In the case of the cylinder, the interpolation function is linear. Such techniques might permit an RG treatment that coarse grains the degrees of freedom of the surface. After sufficient RG iterations, only a few degrees of freedom will remain and an analytic solution could be possible (cf Berker 1979).

Asymmetric amphiphiles seem likely candidates for breaking up down symmetry. If a general expansion around this broken symmetry point also yielded non-neglectable powers of the Gaussian curvature, this might give minimum shapes without the ideal necks that allow buds to pinch off. The divergent saddle curvature of such ideal necks only vanish for terms composed of the mean curvature.

Several writers point out that the Gaussian rigidity cannot be easily measured, except via topology changes. We note that during budding, the system's total Gaussian curvature increases:

$$\frac{1}{2\pi}\oint K dA = V - E + F = \chi = 2(p - g)$$

(cf Kamien 2002) where $K$ is the Gaussian curvature, $V$, $E$, and $F$ are the number of vertices, edges, and faces of a lattice on the surface, $\chi$ is the Euler characteristic, $g$ is the number of handles, and $p$ is the number of separate parts of the system's surfaces.

As buds cleave off, $p$ increases. Perhaps this offers a means of measuring $\overline{\kappa}$.

Other forms of topological defects arise in the interaction of motor proteins and microtubules (Lee 2000). Unlike disclination defects, which look like points in the continuum model of a membrane, these defects have physical extent. Could the interaction of these extended defects with membrane curvature drive or moderate budding? Such coupling between the cytoskeleton and curvature of the membrane could lead to a more complete understanding of division plane placement and dynamics in both prokaryotes and eukaryotes mitosis.

Acknowledgements are due to Mehran Kardar for his helpful guidance and conversations, and also to Vincenzo Vitelli and Roya Zandi for interesting discussions.

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