MATHEMATICAL MODELS OF MEMBRANES

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Abstract. This paper outlines molecular dynamics simulations and their limitations when modeling large-scale shape dynamics of cellular membranes. We explore concepts from differential geometry and cellular-mechanics to describe cellular membranes in terms of Helfrich's equation of generalized shape energy, which provides the theoretical framework for understanding shape changes in liposome vesicles. We present an outline of the derivation of Helfrich's shape equation in order to illustrate how these equations can be used to model large-scale shape changes in biological cells. We conclude by discussing biological applications of the equations in modeling red blood cell shape.

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1. Introduction

The behavior of biological systems, such as lipid bilayers and cellular membranes, can be fundamentally tied to mathematical concepts through theoretical models. Scientists have been using theoretical models to develop a better understanding of biological systems. Computational simulations and free energy functionals utilize mathematics to define abstract physical concepts into concrete mathematical expressions and help us understand how components of the biomolecular systems interact with each other. By solving these expressions, we may model overall membrane dynamics, including cell movement and undulations in cell shape. In this

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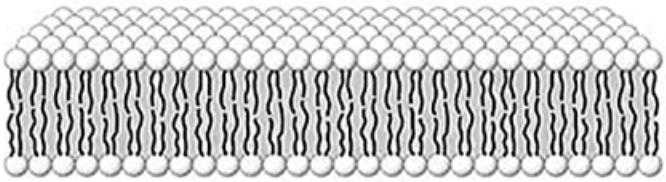
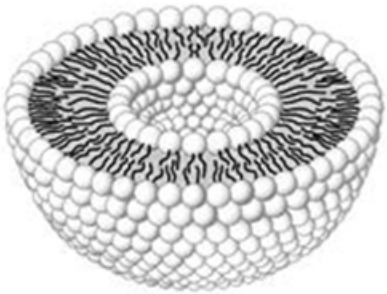
present work, we focus on how molecular dynamics simulations and Helfrich's equation of generalized shape energy use mathematics to depict the dynamics of lipid bilayer systems, and how they have been used to explain biomolecular phenomena associated with cellular membranes such as the shape of human red blood cells.

In general, cellular membranes consist primarily of phospholipid molecules, a type of fatty, biological molecule commonly referred to as a lipid. Lipids are amphipathic molecules, with hydrophilic phosphorous containing head regions and long hydrophobic hydrocarbon tails. In aqueous mediums, the head groups of the lipids aggregate to form the exterior surfaces of the bilayer since they are attracted to water, and act to shield the hydrophobic tails in the bilayer's interior. Bilayer thickness ranges typically from five to eight nanometers thick.



Phospholipid bilayer

Although lipids form the majority of a cell membrane, other molecules such as proteins and cholesterol are interspersed throughout the bilayer and are also attached to its surfaces. Modeling lipid bilayers offers realistic simulations of membrane dynamics, but the effects of these additional structural components must be considered when generalizing properties of the lipid bilayer to cellular membranes. We will see how the study of bilayer dynamics has applications in modeling the shape behavior of a larger group of bilayer systems, liposome vesicles. These vesicles are spherical bilayers, which form spontaneously to completely seal the enclosed environment. Their diameters range from 200 angstroms to 10 micrometers.



Liposome

Different temporal and spatial scales of the membrane system can be accessed and studied by utilizing different simulation and modeling techniques. All-atom molecular dynamics (MD) method depict bilayers at the atomic level, offering simulations of overall dynamical behavior such as bilayer undulations based on explicitly defined atoms and atomic interactions. These simulations are useful for depicting very detailed atomic scale phenomena such as lipid diffusion and intermolecular relationships. Additionally, MD simulations are used to estimate values for intrinsic

properties of cell membranes, such as membrane elasticity, as direct experimentation with lipid bilayers is difficult or impossible, due in part to their microscopic size and limitations of current experimental technology.

Free energy functionals, which are continuum representations of physical behavior in the system of interest, can be utilized to describe larger scale dynamics of membranes, such as the shape behavior of liposome vesicles. In this work, we will focus on functionals that model the effects of stresses on the shape of the liposome. These models are formulated based on fluctuations in equilibrium shape influenced by bending, pressure, and volume deformations and the resistance of the membrane to those fluctuations. We will see how these continuum models offer insights into biological phenomena such as the equilibrium biconcave shape of human red blood cells.

This paper offers a background of MD simulation and their limitations, explaining the necessity of a continuum approach to characterize the shape behavior of larger liposome systems. Before offering a background on the Helfrich equation for shape energy, it discusses methods that are used to describe the bilayer through the language of differential geometry. The paper provides a brief outline of the derivation of the shape equation, subsequently discussing the biological applications of the free energy models and shape equation used in this work.

2. Modeling

2.1. **MD Simulations.** MD simulations utilize the following classical equations of motion to iteratively evolve the movements of a biomolecular system based on the movements of the atoms it contains:

$$F_i = m_i a_i \qquad F_i = -\frac{\partial}{\partial r_i} U$$

The interactions between atoms are pre-defined and are represented by potential energy functions, U as a function of atomic position, r_i. The force acting on each atom, F_i is derived from U by the second equation above. Changes in atomic position and may be calculated by the first equation in small enough windows of time from this force as the initial positions, masses m_i and velocities v_i of each atom are known. Repeating this proscess over small frames of time produces a collection of atomic positions which may be viewed together as a trajectory of the system's movements over time. Trajectories with time lengths of picoseconds to nanoseconds are generally accessible using the current computational resources.

To illustrate this process more clearly, let us consider a flat patch of a lipid bilayer, spanning the x and y directions of a three dimensional box of space with side lengths of 10 micrometers. To simulate a more realistic bilayer system, we will enforce periodic boundary conditions in the x and y directions. In doing so, the simulation box is replicated to form an infinite lattice in these directions, portraying an edgeless bilayer patch. If a molecule leaves the boundaries of the central box in the x or y direction, it will enter through the respective opposite periodic boundary.

Every atom defining the lipid bilayer is represented by a mass and position in the simulation box. From here, we must also define the initial velocities of the atoms and the interactions between them before we begin calculating their movements. The initial velocities of the atoms are obtained by defining the temperature of the simulation, and setting the total momentum of the system, $\Sigma m_i v_i$, to zero, which guarantees that there are no external forces on the box. The interactions

between atoms are defined by potential energy functions based on the nature of the interaction. For example, a single covalent bond interaction between two atoms A and B can be represented by the following potential energy functional:

$$U(r_{AB}) = \frac{1}{2}k_{AB}(r_{AB} - r_{AB,eq})^2$$

where $r_{AB} = r_A - r_B$ is the distance between A and B, $r_{AB,eq}$ is the distance between A and B at which U has a minimum, and k_{AB} is a constant dependent on the identity of the atoms involved. In this interaction, the bond interaction is represented as a spring and models the potential energy characteristic of a simple harmonic oscillator; the further the atoms are apart, the more the string is stretched the more potential energy it has and wants to snap back to its original position.

After interactions between and the initial positions and velocities of the atoms are defined, simulation begins by calculating the force acting on each atom, where this force is the negative derivative of the potential energy function describing the interactions which the atom is involved in. From the force, each atoms velocity for the next timestep can be found by integrating and solving Newtons second equation of motion, $F_i = m_i a_i$, as acceleration is the derivative of velocity and mass is defined.

In small enough windows of time ranging from femto- to picoseconds, new atomic position can be generated based on the calculated velocities. The length of these *timesteps* is chosen to ensure the distances traveled by the atoms are no greater than the length of the shortest bond in the system to ensure realistic atomic dynamics. If the timestep was longer, atoms contained in the shortest bond could move unrealistically outside the small bond length, resulting in a large restoring force acting to counter this relatively large bond "stretch" away from its equilibrium length. Additionally, timesteps are chosen to be smaller than the shortest vibrational wavelength of a bond, as steps too large could gloss over important dynamical information happening at these smaller wavelengths.

By repeating the calculations described above for subsequent timesteps, snapshots of the atoms' positions as a function of timesteps can compiled into trajectories. These trajectories model fluctuations and dynamics of the bilayer as a whole by describing the movements of its atoms, with bilayer phenomena such as undulations appearing on scales of several nanoseconds.

2.2. **Continuum Models.** Computational limitations can be prohibitive when all-atom MD simulations are used to model large liposome membranes, which may require solving for millions of degree of freedom. A modest sized, 100-nanometer liposome consists of tens of thousands of lipid molecules, with each lipid consisting of up to one hundred and fifty atoms. Even if the configuration and the vast amount and variety of interactions between the millions of atoms contained in the liposome were defined, the computational power required to perform the associated calculations would take months to generate even a modest trajectory.

To realistically model and study the behavior of larger bilayer systems, they must be simplified without losing relevant information. In Helfrich's Equation of Generalized Shape Energy, the overall shape of an entire liposome is not modeled as a function of small scale changes in the millions of atoms it contains, but utilizes larger scale membrane properties to parameterize a continuous scalar property of the system: free energy.

Parameters of this free energy model generalize physical characteristics and properties associated with the lipid bilayer system and together offer a description of the relative stability of a membrane in a particular physical configuration. Many of these parameters can be expressed mathematically, utilizing the language of differential geometry. Other parameters are constants quantifying the relative intensity of intrinsic properties of the bilayer, such as elasticity, and may be approximated from analysis of MD simulations. Through outlining the derivation of the shape equation, we will see how Helfrich's equation mathematically models the equilibrium shape and dynamics of the liposome, as they both seek a shape configuration that minimizes the liposome's free energy.

Before discussing the free energy model and how it models liposome shape, we must first become familiar with the bilayer system as a mathematical surface, utilizing differential geometry to describe the physical conformations its surface may take.

2.3. **Curves, Orientation, and Curvature.** Curves and surfaces can be expressed *implicitly* or *explicitly*, but for this discussion we will focus on their *parametric* representation.

Definition 2.1. A smooth parameterized **plane curve** is a subset of $\mathbb{R}^2$ described by the positional vector $\vec{r} : [a, b] \to \mathbb{R}^2$ and scalar value $t \in [a, b]$ such that the position of the curve in $\mathbb{R}^2$ may be represented as functions of t:

$$\vec{r}(t) = (x(t), y(t))$$

where x and y are continuous and have continuous derivatives for all values of $t \in (a, b)$ A plane curve is said to be of class r if x and y have continuous derivatives up to order r, inclusive.

Definition 2.2. A smooth parameterized **space curve** is subset $\mathbb{R}^3$ described by the positional vector $\vec{r}$: $[a, b] \to \mathrm{R}^3$ and scalar value $t \in [a, b]$ such that position of the curve may be represented as functions of t:

$$\vec{r}(t) = (x(t), y(t), z(t))$$

where the functions x, y and z are continuous and have continuous derivatives for all values of $t \in (a, b)$ A space curve is said to be of class r if x, y and z have continuous derivatives up to order r, inclusive.

As t increases from a to b, the positional vector traces out a path of points in space, which, when taken together, form the curve. If the parameter of the curve is taken to be the arc length of the curve, it is said to have a *natural parameterization*, and we will denote the arc length parameter as s. Planar curves may also exist in $\mathbb{R}^3$, having at least one constant parameterization for its coordinates.

At any point along its parameterization, the orientation of a curve may be described by three mutually orthogonal unit vectors called the unit *tangent*, unit *main normal*, and *bi-normal* vectors. We find the vectors of a naturally parameterized curve by differentiating the curve's parameterization with respect to arc length, s.

Definition 2.3. The unit **tangent vector** $\vec{t}(s)$ of a naturally parameterized curve $\vec{r}(s)$ is

$$\vec{t}(s) = \frac{\vec{r}\,'(s)}{||\vec{r}\,'(s)||}$$

Definition 2.4. The unit **main normal** vector $\vec{m}(s)$ of a naturally parameterized curve described by $\vec{r}(s)$ is

$$\vec{m}(s) = \frac{\vec{t}\,'(s)}{||\vec{t}\,'(s)||} = \frac{\vec{r}\,''(s)}{||\vec{r}\,''(s)||}$$

Definition 2.5. The **bi-normal** $\vec{b}(s)$ vector of a naturally parameterized curve described by $\vec{r}(s)$ is

$$\vec{b}(s) = \vec{t}(s) \times \vec{m}(s)$$

The triple vectors define a moving frame and a local coordinate system at each point along the curve. This frame is referred to as the *Frenet Frame* with the *osculating plane* defined by $\vec{m}$ and $\vec{t}$. Properties and characteristics of curves can be described by changes in these vectors and this frame, geometrically symbolizing the relative strengths in which the curve is bent and twisted in space.

The tangent vector has a simple geometric interpretation. The vector $\vec{r}(s+\Delta s)$ - $\vec{r}(s)$ offers the directionality from $\vec{r}(s)$ to $\vec{r}(s+\Delta s)$. By dividing this vector by changes in arc length Δs , and taking the limit as Δs goes to zero, we converge at a finite magnitude vector describing the directionality of the curve at a point: the tangent vector:

$$\lim_{\Delta s \to 0} \frac{\vec{r}(s+\Delta s) - \vec{r}(s)}{\Delta s} = \vec{r}\,'(s)$$

The unit tangent vector is found by normalizing the magnitude of the tangent vector.

Changes in the tangent vector are used to characterize the *curvature* of a curve, and offer a scalar value representing how much the curve bends, or the relative extent to which a curve in not contained in a straight line. Curvature intuitively leads us to the normal vector, as this vector describes the relative rate of the change of tangent vector along the curve.

Definition 2.6. The **curvature** $k(s)$ of curve at a point s along its parameterization is defined as

$$k(s) = ||\vec{t}\,'(s)|| = \frac{1}{\gamma}$$

where γ is the radii of the circle tangent to the curve at that point, known as the osculating circle.

Curvature can be also quantified by the angular speed of rotation of a curve's main normal vector as a function of s. Let $\vec{r}(s)$ describe a plane curve. As we move from point $P = \vec{r}(s)$ to point $Q = \vec{r}(s+\Delta s)$ the tangent vectors at those points, $\vec{r}\,'(s)$ and $\vec{r}\,'(s+\Delta s)$ and the vector describing their difference, $\vec{r}\,'(s+\Delta s)$ - $\vec{r}\,'(s)$ form an isosceles triangle, following from the fact that $\vec{r}\,'(s)$ and $\vec{r}\,'(s-\Delta s)$ are both vectors of the same unit length. Let us denote $\Delta\Phi$ as the angle between $\vec{r}\,'(s+\Delta s)$ and $\vec{r}\,'(s)$. It follows that the length of the third side of the triangle, $\vec{r}\,'(s+\Delta s)$ - $\vec{r}\,'(s)$ is related to the curvature in the following way as Δs goes to zero:

$$||\vec{r}\,'(s+\Delta s) - \vec{r}\,'(s)|| = \Delta\Phi \cdot 1 = \Delta\Phi = ||\vec{r}\,''\Delta s||$$

Thus, the curvature can also be represented as:

$$k(s) = ||\vec{r}\,''(s)|| = \lim_{\Delta s \to 0} |\frac{\Delta\Phi}{\Delta s}|$$

For space curves, another means of curvature must be defined, as the curve not only bends but twists in space. This twisting is measured by the curve's *torsion*. The torsion quantifies the extent to which curve deviates from the osculating plane.

Definition 2.7. The **torsion** $\tau(s)$ of a naturally parameterized space curve at a point s along its parameterization is defined as

$$\tau(s) = -\vec{m}(s) \cdot \frac{d}{ds}\vec{b}(s)$$

Geometrically, the torsion characterizes the angular speed of rotation of the bi-normal vector as a function of s and may be expressed as:

$$\tau(s) = \lim_{\Delta s \to 0} |\frac{\Delta \varphi}{\Delta s}|$$

where $\Delta\varphi$ is the angle between the bi-normal vectors $\vec{b}(s + \Delta s)$ and $\vec{b}(s)$

2.4. **Surfaces.** The lipid bilayer can be represented as a smooth, two dimensional surface embedded in a three dimensional Euclidean space.

Definition 2.8. A **smooth surface** in $\mathbb{R}^3$ is a subset $S \subset \mathrm{R}^3$ such that each point in S has a neighborhood $U \subset S$ and is described by a map $\vec{r}\colon V \to \mathbb{R}^3$ from an open set $V \subset \mathrm{R}^2$ such that

- V and U are homeomorphic, meaning $\vec{r}\colon V \to U$ is a bijection that continuously maps V into U, and its inverse function, $\vec{r}^{\,-1}$ exists and is continuous.
- $\vec{r}(u, v) = (x(u, v), y(u, v), z(u, v))$ has continuous partial derivatives of all orders.
- For all points in S, $\vec{r}$ is a regular parameterization, meaning the first partial derivatives $\vec{r}_u$ and $\vec{r}_v$ are linearly independent.

Before continuing, it is convenient to notate the partial derivatives of a smooth surface described by $\vec{r}(u, v)$ in the following manner:

$$\vec{r}_v = \frac{d\vec{r}}{dv}, \quad \vec{r}_{uu} = \frac{d^2\vec{r}}{du^2}, \quad \vec{r}_{uv} = \frac{d^2\vec{r}}{dudv}, \text{ and } \vec{r}_{vv} = \frac{d^2\vec{r}}{dv^2}$$

We may describe the orientation of a surface at a point by generalizing the orientation of the curves passing through that point. We define a tangent plane at a point on the surface by generalizing the tangent vectors of the curves on a surface through that point and we specify a new surface normal vector with respect to the tangent plane.

Definition 2.9. The **tangent space** of a smooth parameterized surface S at point $p = \vec{r}(u, v)$ contains all the tangent vectors of curves through that point. This space defines a *tangent plane* to that point on a surface.

Definition 2.10. The unit **surface normal** $\vec{n}$ of a smooth parameterized surface at a point $p = \vec{r}(u, v)$ is the vector orthogonal to the tangent plane at point p:

$$\vec{n} = \frac{\vec{r_u} \times \vec{r_v}}{|\vec{r_u} \times \vec{r_v}|}$$

2.5. **Surface curvature.** As with surface orientation, a natural way to investigate how much a surface curves at a point is to look at the curvature of the curves passing though that point on the surface. We relate the curves' curvature to the surface's by projecting the curvature of specific curves into regions described by the Darboux frame.

Definition 2.11. The **Darboux frame** at a point p on a smooth surface with respect to a smooth curve on the surface passing through p, $\vec{r}(s) = \vec{r}(u(s), v(s))$, is defined by the surface normal vector $\vec{n}$, unit tangent vector $\vec{t}$ of the curve, and a *tangent normal* vector $\vec{g} = \vec{N} \times \vec{t}$, which is orthogonal to the surface normal and the curve's tangent vector.

The vectors in this plane are perpendicular to each other, and the frame forms an orthonormal basis for $\mathbb{R}^3$. It follows that the smooth curve, $\vec{r}$ can be written as linear combination of the vectors in this frame.

Definition 2.12. The **normal curvature** κ_n and **geodesic curvature** κ_g of a smooth surface S at point p in the direction specified by the unit tangent, $\vec{t}$ and main normal, $\vec{m}$ vectors of a smooth curve $\vec{r}(s) = \vec{r}(u(s), v(s))$ on S at p are defined by

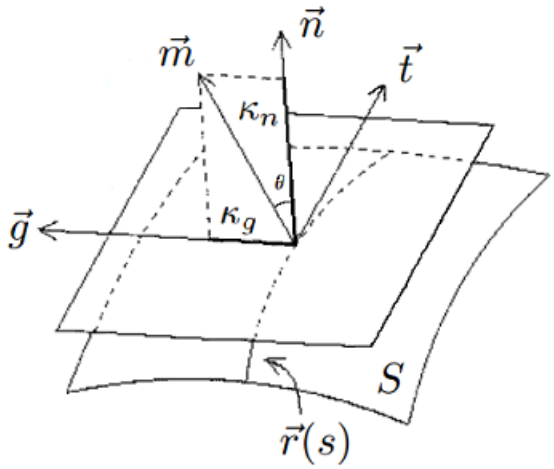
$$\kappa_n(s) = \vec{m}(s) \cdot \vec{n}(s)$$
$$\kappa_g(s) = \vec{m}(s) \cdot \vec{g}(s)$$

and are related to the curve's curvature κ by

$$\kappa \cdot \vec{n} = \kappa_n + \kappa_g$$

κ_n is positive if $\vec{r}(s)$ bends towards the surface normal vector $\vec{n}$, and κ_g is positive if $\vec{r}(s)$ bends towards the tangent normal vector $\vec{g}$.

Geometrically κ_n measures the magnitude of the projection of curvature of the curve, κ onto the surface normal, $\vec{n}$, at that point. Similarly, k_g measures the magnitude of κ projected onto the tangent vector. In this way, κ_n describes the component of the curve's curvature, κ in the surface normal direction of the Darboux frame, while κ_g describes the curvature in the tangent normal direction.



Definition 2.13. Given a naturally parameterized smooth curve $\vec{c}: s \to S(u(s), v(s))$ on a smooth surface S, **geodesic torsion** τ_g at a point p is given by:

$$\tau_g = \tau - \frac{\delta\theta}{\delta s}$$

where τ is the torsion of c at p, and θ is the angle between surface normal and the principal normal of c at s.

The geodesic torsion describes the rate of change of the surface normal around the curve's tangent vector, giving us another means of describing the extent of surface curvature by looking at its curves.

Definition 2.14. The **normal section of the surface** at a point p along a direction specified by a vector $\vec{x}$ is the plane generated by and containing the surface normal at that point, $\vec{n}$, and $\vec{x}$.

Definition 2.15. Principal curvatures, c_1 and c_2 of a surface S at point p are the extremal values of k_n of the smooth curves generated by the intersection of the normal section of the surface with S. The directionality of the normal section is given by the curves' tangent vectors, and called the **principal directions**. c_1 is taken as the maximum and c_2, the minimum.

Definition 2.16. The **Gaussian curvature**, K at a point p of a surface is defined as the product of the principal curvatures:

$$K = c_1 c_2$$

Definition 2.17. The **mean curvature** H at a point p of a surface is defined as

$$H = \frac{c_1 + c_2}{2}$$

2.6. **The First Fundamental Form.** Describing surface curvature more rigorously requires a discussion of the first and second fundamental forms of a surface. These forms define important metric properties of a surface and can be used to describe curvature in terms of how much Euclidean length and volume is deformed at points on a surface. The first fundamental form acts as the surface metric, enabling calculations of lengths and area on surface as it defines a unit of length on the surface.

Definition 2.18. Let p be a point on a smooth surface S. The **first fundamental form** at p is defined as

$$I = < \vec{V}, \vec{U} >$$

where $\vec{V}$ and $\vec{U}$ are elements of the tangent space at p.

Since the inner product on $\mathbb{R}^3$ is defined as the dot product, the first fundamental form may be expressed in quadratic from:

$$Edu^2 + 2Fdudv + Gdv^2$$

where the coefficients of the first fundamental form, E, F, and G are

$$E = \vec{r}_u \cdot \vec{r}_u, \quad F = \vec{r}_u \cdot \vec{r}_v, \quad \text{and,} \quad G = \vec{r}_v \cdot \vec{r}_v$$

We will see later that it is also convenient to express the coefficients of the first fundamental form in a matrix:

$$I_{u,v} = \begin{vmatrix} E & F \\ F & G \end{vmatrix}$$

denoting its determinant as $|g|=EG-F^2$

We now will see how the first fundamental form defines the *surface metric* on a surface by considering its relation to the differential arc length Δs of a smooth parameterized curve on a smooth surface S. The differential arc length defines length on the surface metric by defining the the arc length between two infinitely close points of a curve on a surface.

Definition 2.19. The **differential arc length** Δs of a smooth curve, $\vec{r}(t)$ is the distance along the contour of the curve as $t_1 \to t_2$, where t_1 and t_2 are parameters used to describe points of the curve.

The differential arc length may be approximated by the Euclidean distance, or chord length, between two points of the curve, $p = \vec{r}(t)$ and $q = \vec{r}(t + \Delta t)$ as Δt goes to zero. To find this infinitely small chord length, we take a first order Taylor expansion of the magnitue of the vector $\Delta\vec{r} = \vec{r}(t) - \vec{r}(t + \Delta t)$ as Δt goes to zero:

$$\Delta s \simeq |\Delta\vec{r}\,| = |\vec{r}(t) - \vec{r}(t + \Delta t)| = |\frac{d\vec{r}}{dt}\Delta t + \frac{1}{2}\frac{d^2\vec{r}}{dt^2}(\Delta t)^2| \simeq |\frac{d\vec{r}}{dt}|\Delta t$$

As p approaches q or Δt goes to zero (utilizing slightly different notation) the length of Δr becomes the differential arc length, ds, of the curve:

$$ds = |\frac{d\vec{r}}{dt}|dt = |\vec{r}\,'|dt = \sqrt{\vec{r}\,' \cdot \vec{r}\,'}dt$$

The first fundamental form shows itself when we compute the differential arc length for the smooth curve $\vec{r}(u(t), v(t))$ on a surface $\vec{r}(u, v)$:

$$\begin{aligned}
ds &= \sqrt{\vec{r}\,' \cdot \vec{r}\,'}dt \\
&= \sqrt{(\vec{r_u}u'(t) + \vec{r_v}v'(t)) \cdot (\vec{r_u}u'(t) + \vec{r_v}v'(t))}dt \\
&= \sqrt{(\vec{r_u} \cdot \vec{r_u})(u'(t))^2 + 2(\vec{r_u} \cdot \vec{r_v})u'(t)v'(t) + (\vec{r_v} \cdot \vec{r_v})(v'(s))^2}dt \\
&= \sqrt{(E(u'(t))^2 + 2Fu'(t)v'(t) + G(v'(t))^2)}dt \\
&= \sqrt{Edu^2 + 2Fdudv + Gdv^2} \\
&= \sqrt{I}
\end{aligned}$$

Thus the first fundamental form encodes information about the surface metric by describing small changes in length on a surface as a function of small changes in the surface's parameterization:

$$ds^2 = I$$

It follows the arc length of a naturally parameterized smooth curve, $r(s) = r(u(s), v(s))$ with values of s ranging from a to b on a smooth surface is:

$$\int_a^b |\vec{r}\,'(s)ds| = \int_a^b \sqrt{(Eu'(s)^2 + 2Fu'(s)v'(s) + Gv'(s)^2)}ds$$

As the first fundamental form describes length on a parameterized surface, it intuitively follows that it is related to area on a surface. Just as the ds offers a description of length by approximating the arc length between two infinitesimally

close points $\vec{r}(u,v)$ and $\vec{r}(u+du, v+dv)$ on a parameterized surface, the area element,

$$dA = |\vec{r}_u \times \vec{r}_v|$$

approximates the area of an infinitesimally small parallelogram with vertices $\vec{r}(u,v), \vec{r}(u+du), \vec{r}(u, v+dv)$ and $\vec{r}(u+du, v+dv)$ and can be used to calculate area of regions on a surface.

Definition 2.20. The **surface area** of a bounded region, $\vec{r}(Q)$ on a smooth parameterized surface, $\vec{r}$ is given by

$$\int\int_Q |\vec{r}_u \times \vec{r}_v|\, du\, dv \;= \int\int_Q dA\, du\, dv.$$

The first fundamental form and dA are related through the determinant of the first fundamental form, $g = EG - F^2$

$$EG - F^2 = (\vec{r_u} \cdot \vec{r_u})(\vec{r_v} \cdot \vec{r_v}) - (\vec{r_u} \cdot \vec{r_v})^2 = |\vec{r}_u \times \vec{r}_v|^2$$

Thus

$$dA = \sqrt{|g|}$$

2.7. **Second fundamental form.** The second fundamental form describes the extent to which a point p on a smooth surface deviates from the tangent plane and can be related to the first fundamental form if we observe how arc length of a surface curve though p changes as p is pushed or pulled along its surface normal vector.

Definition 2.21. The quadratic form of the **second fundamental form** of a surface is expressed as

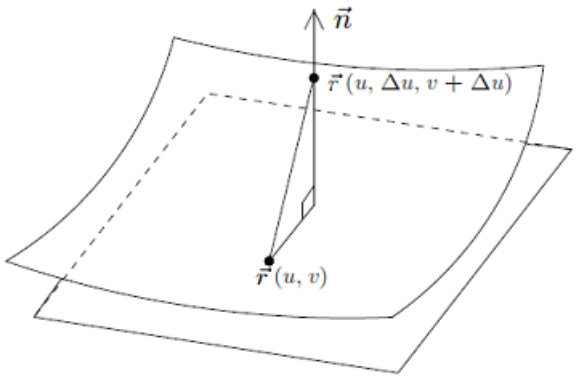
$$Ldu^2 + 2Mdudv + Ndv^2$$

where its coefficients, L, M, N are defined to be the projections of the second partial derivatives of $\vec{r}$ onto the unit surface normal vector, $\vec{n}$:

$$L = \vec{r}_{uu} \cdot \vec{n}, \;\; M = \vec{r}_{uv} \cdot \vec{n}, \;\; N = \vec{r}_{vv} \cdot \vec{n}$$

We will see where this expression comes from by describing the extent to which a point on a smooth surface deviates from its tangent plane. Suppose that $\vec{r}$ describes a smooth surface. As the parameters u and v of $\vec{r}$ change to $u+du$ and $u+dv$, the surface moves away from the tangent plane at $\vec{r}(u,v)$ by a distance of

$$\vec{n}(u,v) \cdot (\vec{r}(u+du, u+dv) - \vec{r}(u,v))$$



By the two variable form of Taylors theorem, $\vec{r}(u+du, u+dv) - \vec{r}(u,v)$ is equal to

$$\vec{r}_u du + \vec{r}_v dv + \frac{1}{2}(\vec{r}_{uu}du^2 + 2\vec{r}_{uv}dudv + \vec{r}_{vv}dv^2) + \text{remainder}$$

where $\frac{\text{remainder}}{du^2+dv^2}$ tends to zero as $du^2 + dv^2$ tends to zero. As $\vec{r}_u$ and $\vec{r}_v$ are tangent vectors, they are perpendicular to $\vec{n}(u,v)$ thus the deviation of the surface from its tangent plane may be expressed as

$$\frac{1}{2}(Ldu^2 + 2Mdudv + Ndv^2) + remainder$$

The second fundamental form can also be expressed in a matrix, where:

$$II_{uv} = \begin{vmatrix} L & M \\ M & N \end{vmatrix}$$

The relation of the second fundamental form to the first can be seen by considering changes in the surface metric, or first fundamental form, as is a point is deformed about the surface normal. Suppose we have a surface S described by the parameterization $\vec{r}(u,v)$. Deformational changes in this surface along the surface normal, $\vec{n}$ can be described by a family of surfaces, $\vec{R}(u,v,t) = \vec{r}(u,v)$ - $t\vec{r}(u,v)$, where the first fundamental form of $\vec{R}$ is expressed as

$$I_R = E(t)du^2 + 2F(t)dudv + G(t)dv^2$$

where $\vec{R}_u = \vec{r}_u - t\vec{n}_u$, $\vec{R}_v = \vec{r}_v - t\vec{n}_v$. The second fundamental form of $\vec{r} = \vec{R}(u,v,0)$ is found to be

$$II_R = \frac{1}{2}\frac{d}{dt}I_R$$

It should be noted that this expression corresponds to the second fundamental form only for the surface $\vec{r}(u,v) = \vec{R}(u,v,0)$ and not of the entire family of surfaces $\vec{R}(u,v,t)$ as we considered the change in arc length deformation expressed by $\frac{1}{2}\frac{d}{dt}I_R$ only at time t=0 along the normal. Derivatives at different points correspond to the second fundamental form of different surfaces in the family $\vec{R}(u,v,t)$.

2.8. **Normal, Principal, Mean, and Gaussian Curvature.** The curvature of a surface can be expressed in terms of the first and second fundamental forms of a surface.

Theorem 2.22. *If $\vec{r}$:$(u,v) \to \mathbb{R}^3$ describes a smooth surface S and $\vec{r}(s) = \vec{r}(u(s), v(s))$, a smooth curve on S at point p, then the normal curvature, κ_n is given by*

$$\kappa_n = \frac{II_r}{I_r} = \frac{L + 2M\Lambda + N^2}{E + 2F\Lambda + G\Lambda^2}$$

where $\Lambda = \frac{du}{dv}$ is the direction of the tangent line at $\vec{r}(s)$

Proof. Let $\vec{r}$:$(u,v) \to \mathbb{R}^3$ describe a smooth surface S, and $\vec{r}(s) = \vec{r}(u(s), v(s))$ a smooth curve on S through point p. We know by definition $\vec{n} \cdot \vec{t} = 0$, and by differentiating this expression along the curve with respect to s, we obtain

$$\frac{d\vec{t}}{ds} \cdot \vec{n} + \vec{t} \cdot \frac{d\vec{n}}{ds} = 0$$

Thus, we arrive at the following expression for the normal curvature at s by definition of the triple unit vectors, ds and the first fundamental form:

$$\kappa_n(s) = \vec{m}(s) \cdot \vec{n}(s) = \frac{d\vec{t}}{ds} \cdot \vec{n} = -\vec{t} \cdot \frac{d\vec{n}}{ds} = -\frac{dr}{ds} \cdot \frac{dn}{ds} = -\frac{dr \cdot dn}{dr \cdot dr}$$
$$= \frac{Ldu^2 + 2Mdudv + Ndv^2}{Edu^2 + 2Fdudv + Gdv^2}$$

where

$$L = -\vec{r}_u \cdot \vec{n}_u \quad M = -\frac{1}{2}(\vec{r}_u \cdot \vec{n}_v + \vec{r}_v \cdot \vec{n}_u) = -\vec{r}_u \cdot \vec{n}_v = -\vec{r}_v \cdot \vec{n}_u \quad N = -\vec{r}_v \cdot \vec{n}_v$$

and E, F and G are the coefficients of the first fundamental form. Since $\vec{r}_u$ and $\vec{r}_v$ are perpendicular to $\vec{n}$ we may write an alternative expression for L, M and N corresponding to the coefficients of the second fundamental form: we have $\vec{r}_u \cdot \vec{n} = 0$ and $\vec{r}_v \cdot \vec{n} = 0$.

$$0 = (\vec{r}_u \cdot \vec{n})_u = \vec{r}_{uu} \cdot \vec{n} + \vec{r_u} \cdot \vec{n_u} \implies L = -\vec{r}_{uu} \cdot \vec{n}$$

$$0 = (\vec{r}_v \cdot \vec{n})_v = \vec{r}_{vv} \cdot \vec{n} + \vec{r_v} \cdot \vec{n_v} \implies [N = \vec{r}_{vv} \cdot \vec{n}$$

$$0 = (\vec{r}_u \cdot \vec{n})_v = \vec{r}_{uv} \cdot \vec{n} + \vec{r_u} \cdot \vec{n_v} \implies M = \vec{r}_{uv} N$$

Therefore, the normal curvature is given by:

$$\kappa_n(s) = \frac{II_r}{I_r} = \frac{L + 2M\Lambda + N^2}{E + 2F\Lambda + G\Lambda^2}$$

□

The above theorem enables us to calculate the extreme values, or principal curvatures, of a smooth surface at point p by evaluating $\frac{d\kappa_n}{d\Lambda}$=0. which gives:

$$(E + 2F\Lambda + G\Lambda^2)(N\Lambda + M) - (L + 2M\Lambda + N\Lambda^2)(G\Lambda + F) = 0 \tag{2.23}$$

Thus

$$\kappa_n = \frac{L + 2M\Lambda + N^2}{E + 2F\Lambda + G\Lambda^2} = \frac{M + N\Lambda}{F + G\Lambda}$$

Furthermore, since

$$E + 2F\Lambda + G\Lambda^2 = (E + F\Lambda) + \Lambda(F + G\Lambda)$$
$$L + 2M\Lambda + N\Lambda^2 = (L + M\Lambda) + \Lambda(M + N\Lambda)$$

We may reduce (2.24) to

$$(E + F\Lambda)(M + N\Lambda) = (L + M\Lambda)(F + G\Lambda)$$

Thus, the extreme values of κ_n must satisfy both

$$(L - \kappa_n E)du + (M - \kappa_n F)dv = 0$$
$$(M - \kappa_n F)du + (N - \kappa_n G)dv = 0$$

Therefor, the principal curvatures c_1 and c_2 are the roots of the following system of equations

$$det(II - cI) = 0 \quad det \begin{vmatrix} L - \kappa_n E & M - \kappa_n F \\ M - \kappa_n F & N - \kappa_n G \end{vmatrix} = 0$$

which may also be expressed in quadratic form as:

$$(EG - F^2)\kappa_n^2 - (NE + LG - 2MF)\kappa_n + (LN - M^2) = 0$$

Expressions of the Gaussian and mean curvatures may be similarly expressed in terms of the first and second fundamental forms:

Theorem 2.24. *The **Gaussian curvature** K of a surface is*

$$K = \frac{LN - M^2}{EG - F^2}$$

Theorem 2.25. *The **mean curvature** H of a surface is*

$$H = \frac{LG - 2FM + NE}{2(EG - F^2)}$$

3. Helfrich's Equation of Generalized Shape Energy

We saw how differential geometry offers a more rigorous means of describing curvature and metric properties of lipid bilayers by representing them as mathematical surfaces. Fluctuations in overall lipid bilayer shape can be represented from a more unified point of by view by associating free energy with deformations due to bending, pressure differences, and surface tension.

The *free energy* of a system may be thought of as its capacity to do work, and can be used to describe the energetic stability of a system. Higher values of free energy are energetically unfavorable, and the system, in this case the liposome membranes, will seek to lower its free energy by moving into a more favorable conformation. The membrane's movement may be thought of as work.

The general equation relating curvature, pressure, and volume deformations of a liposome's shape to free energy is given below:

Definition 3.1. Helfrich's Equation of Generalized Shape Energy describes the free energy of a liposome represented as a smooth parameterized surface, $\vec{r}$ and is given by

$$F_{Helfrich} = \frac{k_c}{2} \oint (2H - c_0)^2 dA + \Delta P \oint dV + \lambda \oint dA$$

where dA is the area element, dV is the volume element, ΔP is the pressure difference between the inside and outside of the liposome, k_c is the bending modulus, λ is the tensile stress acting on the surface, and c_0 is the spontaneous curvature of the membrane, which is the value of mean curvature that minimizes the free energy of the system.

The first part of the equation, $\frac{k_c}{2} \oint (2H - c_0)^2 dA$ expresses the total elastic energy of the membrane, or the free energy associated with bending deformations on the surface of the membrane. We arrive at the *total* elastic energy of the membrane by integrating the elastic energy of curvature per unit area over the surface of the membrane, utilizing the area element dA which we previously discussed.

Definition 3.2. The

elastic energy of curvature per unit area of a membrane F_c is given by

$$F_c = \frac{k_c}{2}(2H - c_0)^2 + \bar{k}K$$

where K Gaussian curvature, k_c is the bending modulus, c_0 is the spontaneous curvature, and $\bar{k}$ is the elastic modulus of Gaussian curvature.

Since we have described the membrane as a smooth mathematical surface, we may describe bending deformations on the membranes in terms of curvature. We see that the elastic energy of curvature per unit area utilizes both mean and Gaussian curvature to represent membrane shape in terms of free energy at a particular point on a membrane. The expression quantifies the extent to which the curvature deviates from the most energetically favorable curvature, or the equilibrium curvature. Doing so describe the relative stability of the liposome's conformation.

The first part of the curvature free energy accounts for energetic differences in mean curvature. It relates the mean curvature to free energy using a functional form similar to one we saw earlier:

$$U(r_{AB}) = \frac{1}{2}k_{AB}(r_{AB} - r_{AB,eq})^2$$

Here however, instead of describing the potential energy associated with stretching the bond length of two atoms from its equilibrium length, we describe the potential energy associated with deviating from the equilibrium value of mean curvature. C_0 represents the mean curvature at which the shape free energy is at its equilibrium value, or the mean curvature a membrane naturally assumes if subjected to no deformations.

The bending modulus, k_c is a constant which quantifies the extent to how much the bending free energy is affected from a deviation from the spontaneous curvature and can be thought of as the rigidity of a membrane. A membrane with a small value for k_c is a flexible membrane since there is a small scaling effect associated with deforming the membrane's curvature from its optimum value. Higher values for k_c result in a more rigid membrane, as higher values causes even the small deformations to be energetically unfavorable. Gaussian curvature contributions to free energy are accounted for by kK, with the elastic modulus of Gaussian curvature, k describing the relative rigidity of the membrane with respect to Gaussian curvature deformations.

Pressure and volume deformations are also accounted for in the general shape equation. $\Delta P \int dV$ describes the free energy associated with volume deformations induced by pressure differences, where ΔP is the Lagrange multiplier due to the constraint of constant volume of the system and denotes the osmotic pressure difference between the inside and outside of the membrane. The volume element, dV at a point on the membrane is expressed in terms of the determinant of the first fundamental form $|g|=EG - F^2$, the surface's parameterization, and the surface normal:

$$dV = \frac{1}{3}\sqrt{g}(\vec{r} \cdot \vec{n})dudv$$

In nature, we see the effects of $\Delta P \int dV$ arising if the liposome in a salty environment. The semipermeable nature of the bilayer allows water but does not readily allow salt atoms to diffuse through the bilayer. In an attempt to equilibrate the difference in salt concentrations, water leaves the membrane's interior, creating an osmotic pressure difference. This pressure difference may be assumed to characterize the extent to which the liposome's volume is reduced in size as water leaves the membrane. Similarly, the final term, λ accounts for free energy associated with deformations induced by surface or other interfacial tensions, where λ this Lagrange multiplier due to the constraint of area.

4. The Shape Equation

An expression for the equilibrium shape of the liposome vesicle is found by minimizing the shape energy of an insignificantly perturbed liposome, which is done by calculating the first variation of Helfrich's equation of generalized shape energy and setting it equal to zero. In this way, the derivation acts to model the dynamics of a liposome, as they both also seek to find the most energetically favorable configuration of the liposome in space. We will outline the derivation the shape equation by presenting the steps for calculating the first variation of $F_{Helfrich}$, denoted $\delta^{(1)}F_{Helfrich}$.

Theorem 4.1. *The **general shape equation** of a liposome vesicle represented by a smooth parameterized surface is denoted:*

$$\Delta p - 2\lambda H + k_c(2H + c_0)(2H^2 - 2K - c_0H) + 2k_c \bigtriangledown^2 (2H + c_0) = 0$$

Where H is surface's mean curvature, k_c is bending modulus, c_0 is spontaneous curvature, K is Gaussian curvature, and $\bigtriangledown$ is the Laplace-Beltrami operator on the surface

Proof. Let $\vec{r} : (u, v) \to \mathbb{R}^3$ describe a smooth surface representing the liposome. We will consider a new surface $\mathbf{r}$ created by slightly deforming $\vec{r}$ with respect to its surface normals:

$$\mathbf{r} = \vec{r} + \Psi \cdot \vec{n}$$

where $\Psi(u, v)$ is a sufficiently small and smooth function representing a slight perturbation to the liposome. We begin calculating the first variation of $F_{Helfrich}$ by calculating the first variation of F_c:

$$\delta^{(1)}F_c = \frac{k_c}{2}\oint (2H - c_0)^2\delta^{(1)}(dA) + \frac{k_c}{2}\oint 4(2H - c_0)^2 dA(\delta^{(1)}H)dA \tag{4.2}$$

One may find the first variation of area element dA, mean curvature H, and volume element dV for $\mathbf{r}$ by writing dA, H and dV in terms of $\vec{r}$ and Ψ. The first order variation gives us $\delta^{(1)}dA$, $\delta^{(1)}dV$ and $\delta^{(1)}H$ as:

$$\delta^{(1)}dA = -2H\Psi\sqrt{g}dudv \tag{4.3}$$

$$\delta^{(1)}dV = \Psi\sqrt{g}dudv \tag{4.4}$$

$$[\delta^{(1)}H = (2H^2 - K)\Psi + \frac{1}{2}g^{ij}(\Psi_{ij} - \Gamma^k_{ij}\Psi_k)]dudv \tag{4.5}$$

where g^{ij} is the inverse of the matrix of first fundamental form of $\vec{r}$, Ψ_{uv} and Ψ_k are defined as:

$$\Psi_{uv} = \frac{d}{du}\Psi \cdot \frac{d}{dv}\Psi$$

$$\Psi_k = \frac{d}{d_k}\Psi$$

where $k = u, v$, and the *Christoffel symbol*, Γ^k_{ij} is used to expand $\vec{r}_{ij}$:

$$\vec{r}_{ij} = \Gamma^k_{ij} \cdot \vec{r}_k + \vec{r}_k \cdot \vec{n}$$

The following expression can be found by plugging (4.3) and (4.4) into (4.2) and simplifying:

$$\delta^{(1)}F_c = k_c \oint [(2H+c_0)(2H^2-c_0H-2K)\Psi+\frac{1}{2}g^{ij}(2H+c_0)\psi_{ij}-g^{ij}\Gamma^k_{ij}(2H+c_0)\Psi_k]g^{1/2}dudv$$

By integrating Ψ_u and Ψ_{uv} by parts, noting the two relations:

$$\oint f\psi_k dudv = -\oint f_k \psi dudv$$

$$\oint f\psi_{uv} dudv = \oint f_{uv}\psi dudv$$

One may obtain:

$$\delta^{(1)}F_c = k_c \oint \left\{(2H+c_0)(2H^2 - c_0H - 2K)g^{1/2} + [g^{1/2}g^{ij}(2H+c_0)]_{ij} + [g^{1/2}g^{ij}(2H+c_0)\Gamma^k_{ij}]_k\right\}\psi dudv$$

This equation can be simplified by considering the following relations:

- $[g^{1/2}g^{ij}(2H+c_0)]_{ij}$ may be rewritten as

$$[g^{1/2}g^{ij}(2H+c_0)]_{ij} = [(g^{1/2}g^{ij})_j(2H+c_0)]_i + [g^{1/2}g^{ij}(2H+c_0)\Gamma^k_{ij}]_k\psi dudv$$

- For functions $f(u,v)$ where $u, v = i, j$ one may prove:

$$[(g^{1/2}g^{ij})_j f]_i = -(\Gamma^k_{ij}g^{1/2}g^{ij}f)_k$$

- If we define the *Laplace-Beltrami operator* on the surface $\vec{r}(u,v)$ as

$$\bigtriangledown^2 = g^{1/2}\frac{\partial}{\partial i}(g^{1/2}g^{ij}\frac{\partial}{\partial j})$$

We can rewrite $[g^{1/2}g^{ij}(2H+c_0)_j]_i$ as

$$[g^{1/2}g^{ij}(2H+c_0)_j]_i = g^{1/2}\bigtriangledown^2(2H+c_0)$$

These relations lead to the following expression for the first variation of F_c:

$$\delta^{(1)}F_c = k_c\oint[(2H+c_0)(2H^2 - c_0H - 2K) + \bigtriangledown^2(2H+c_0)]\psi g^{1/2}dudv$$

Using similar methods, one may find the the first variation of F,

$$\delta^{(1)}F = \delta^{(1)}F_c + \delta^{(1)}(\Delta p\int dV) + \delta^{(1)}(\lambda\int dA)$$

to be

$$\delta^{(1)}F = \oint[\Delta p - 2\lambda H + k_c(2H+c_0)(2H^2 - c_0 - 2K) + k_c\bigtriangledown^2(2H+c_0)]\psi g^{1/2}dudv$$

If $\mathbf{r}$ describes an equilibrium configuration of a vesicle, $\delta^{(1)}F$ must vanish for any significantly small and smooth function Ψ as an equilibrium configuration has shape free energy at a minimum value. This minimization condition of $\delta^{(1)}F$ leads to the following expression for shape free energy:

$$\Delta p - 2\lambda H + k_c(2H+c_0)(2H^2 - c_0H - 2K) + 2k_c\bigtriangledown^2(2H+c_0) = 0$$

□

5. Biological Applications: Red blood Cells

The shape equation is a fourth order highly non-linear partial differential equation and finding any non-trivial solution is not an easy task. Deuling and Helfrich [5] have found several particular solutions by assuming the vesicles are surfaces of revolution, reducing the shape equation to a third order non-linear ordinary differential equation, which may be further simplified and expressed as a second order ordinary differential equation upon integration. Under additional restraints to pressure and surface tension, this simplified equation offers solutions agree well with unique biconcave disc shape of normal human red blood cells, which may be considered as liposome vesicles as they do not contain nuclei in their mature form. Below we outline Deuling and Helfrich's derivation of a simplified shape equation.

The simplified parameterization of the red blood cell is given by the plane curve $z : x \to \mathbb{R}^2$. Only positive values of x are considered as the z axis is taken to be the cell's axis of rotational symmetry. In this parameterization, the principal curvatures are along the cell's meridians, c_m and parallels of latitude, c_l and expressed:

$$c_p = \frac{\sin\psi}{x} \tag{5.1}$$

$$c_m = \frac{d\psi}{dx}\cos\psi \tag{5.2}$$

and the surface contour obtained by integration of the first order equation:

$$\frac{dz}{dx} = -\tan\psi$$

where ψ denotes the angle between the rotational axis, z, and the surface normal of the cell.

The contour for which the total elastic energy is minimal at a given volume and surface area may be found by solving the respective shape equation for this system:

$$\delta^{(1)}[(\frac{1}{2}k_c\int(c_m + c_p - c_0)^2 dA + \Delta p\int dV + \lambda\int dA] = 0 \tag{5.3}$$

Expressing dV and dA as

$$dV = \pi \cdot x^3 \cdot c_p(1 - x^2 \cdot c_p^2)^{-1/2}dx$$

$$dA = 2 \cdot \pi \cdot x^3 \cdot (1 - x^2 \cdot c_p^2)^{-1/2}dx$$

and utilizing the relation obtained from combining (5.1) and (5.2):

$$\frac{dc_p}{dx} = \frac{(c_m - c_p)}{x}$$

to eliminate c_m gives a new version of (5.3):

$$\delta\int_0^{x_m} x(1 - x^2 \cdot c_p^2)^{-1/2} \cdot [[x(dc_p/dx) + 2 \cdot c_p - c_0]^2 + (\Delta p/k_c) \cdot x^2 \cdot c_p + 2\lambda/k_c]dx = 0$$

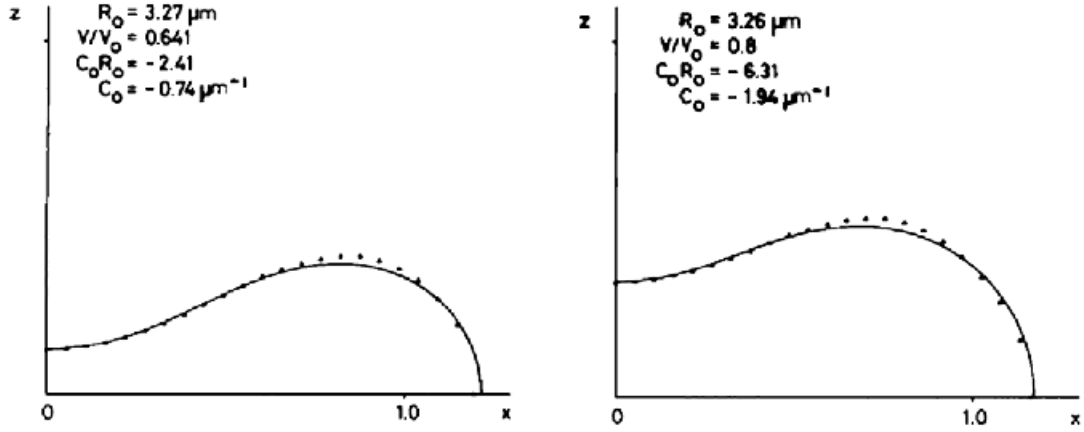
where $c_p(x)$ is the function to be varied. Preforming the variation gives us:

$$\frac{dc_p}{dx} = x \cdot (1 - x^2 \cdot c_p^2)^{-1} \cdot [(\frac{1}{2})c_p[(c_p - c_o)^2 - c_m^2] + \frac{\lambda}{k_c} \cdot c_p + (\frac{1}{2}) \cdot \Delta p/k_c] - \frac{(c_m c_p)}{x}$$

This equation may be solved numerically resulting in a relation which yields the contour, $z(x)$, upon a further integration:

$$z(x) - z(0) = -\int \tan\tau dx = -\int x \cdot c_p \cdot (1 - x^2 \cdot c_p^2)^{-1/2} dx$$

Under these assumptions, it was found that this method offers numerical solutions that agree well with experimentally derived contours of normal human red blood cells determined by interference microscopy. By modeling the existing shape of red blood cell membranes, the Helfrich theory offers motivations to why red blood cells assume their unique circular biconcave discoid shape in their natural state. Below we attach selected examples as presented in [5]. We can see that the solid contour lines obtained from the shape equation closely match those determined from interference microscopy, which are represented by the dotted contours.



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