Structure, shape and dynamics of biological membranes.

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CHAPTER 2

DIFFERENTIAL GEOMETRY

Differential geometry is the branch of mathematics that studies geometrical objects in an analytical way, using differential and integral calculus. In this chapter we introduce the differential geometry of curves and surfaces, and apply them to biopolymers and biomembranes. We discuss Gauss's Theorema Egregium and the Gauss-Bonnet Theorem and their implications. We also introduce the Canham-Helfrich free energy which will allow us to calculate the minimal-energy shapes of biomembranes.

2.1 Manifolds

Differential geometry is the branch of mathematics that studies geometrical objects in an analytical way, using differential and integral calculus. Its techniques and results are applicable to many problems in biophysics, and it is particularly suited to describe the behavior of polymers and membranes in three-dimensional space. In the language of differential geometry, we will consider these as one- and two-dimensional manifolds embedded in two- or three-dimensional flat Euclidean space. A manifold is a mathematical object that has the property that around any of its points it is locally flat, although it may be curved and close upon itself on large scales. Locally, an n-dimensional manifold therefore looks like $\mathbb{R}^n$, and we can parametrize it in a local coordinate system $\{x_i\}_{i=1,\ldots,n}$. If there is another point nearby, with another coordinate system $\{y_i\}_{i=1,\ldots,n}$, then there is a continuous bijection between the two in the region where they overlap. On a smooth manifold all such bijections are smooth maps (*i.e.*, if both the map itself and its inverse are infinitely differentiable).

In this chapter we will introduce the differential geometry of curves and surfaces. Both biopolymers and biomembranes have a sufficiently large aspect ratio that they can effectively be described as one- and two-dimensional objects respectively. Unlike for example a soap film, another example of an effectively two-dimensional object, the molecular structure of the polymers and lipid bilayers does have an effect on the total energy of the manifolds. In particular there will be effects on the bending of the manifolds, which are reflected in the curvature energy. It is not known whether biological membranes are smooth or not, or in other words whether nature 'allows kinks' or not. However, there are clearly possibilities to induce kinks, for example by the inclusion of wedge-shaped proteins. Boundaries within the membrane where the physical parameters change are another example. We will consider the membrane to be a smooth manifold within any region for which the physical parameters are the same, and pay particular attention to such boundaries and inclusions.

Although manifolds are mathematical objects by themselves that can exist and be described without the need of any embedding space, in our three-dimensional reality the embedding space does play a role. Some properties of the manifold are intrinsic and therefore the same whichever embedding space we choose, but unfortunately the curvature does not satisfy that condition. We need to make explicit reference to the space in which we see the manifold, and therefore we distinguish between curves in $\mathbb{R}^2$ and curves in $\mathbb{R}^3$. In the case of the membrane there are two different curvatures, one of which is intrinsic, but the other one is not. As we will see in section 2.3.5, for creatures like cells living in the embedding space, the most important curvature in terms of energy contributions will be the extrinsic one, defined only in the larger Euclidean space that is its home.

There is a vast literature on differential geometry, both in the context of

pure mathematics and in the connection with physics. For a thorough introduction into manifolds, including proofs of the theorems in sections 2.3.3 and 2.3.4, see *e.g.* Millman and Parker [33], Spivak [34] or Do Carmo [35]. For an excellent overview of applications of differential geometry to biopolymers and biomembranes, of which many results are used in this chapter, see Kamien [36].

2.2 Differential geometry of curves

2.2.1 Curves in the plane

Since a curve is a one-dimensional object, we can label its points by a single parameter t, running over a real interval $[a, b]$. If we choose a coordinate system for the embedding space $\mathbb{R}^2$, the coordinates of the point labelled by a given value of t can be written as $\vec{r}(t)$. If our curve represents a polymer, and we are interested in the spatial conformation of that polymer, we will want to associate an energy with every possible conformation. As mentioned above, that requires that we consider the curvature of the polymer. In principle we could do that with the description in terms of $\vec{r}(t)$, but our calculations will be significantly simplified by choosing the arc length s as the parameter to measure the length along the curve. The arc length will run from 0 at $\vec{r}(a)$ to L, the length of the curve, at $\vec{r}(b)$. To find an expression for the arc length, we therefore first need to calculate the total length L of the curve. For an infinitesimal parameter step dt, the length of the curve between t and $t + dt$ is given by

$$\left|\left| \lim_{dt\to 0} \frac{\vec{r}(t+dt) - \vec{r}(t)}{dt} \right|\right| = \left|\left| \frac{\mathrm{d}\vec{r}(t)}{\mathrm{d}t} \right|\right|, \tag{2.1}$$

so we can find L by integrating the norm of the tangent vector $\frac{d\vec{r}(t)}{dt}$ to the curve over the interval $[a, b]$:

$$L = \int_a^b \sqrt{\frac{\mathrm{d}\vec{r}(t)}{\mathrm{d}t} \cdot \frac{\mathrm{d}\vec{r}(t)}{\mathrm{d}t}}\, \mathrm{d}t. \tag{2.2}$$

Since the arc length measures distance along the curve, we can simply calculate it from the arbitrary parametrization $\vec{r}(t)$ by calculating the distance from the starting point:

$$s(t) = \int_a^t \sqrt{\frac{\mathrm{d}\vec{r}(u)}{\mathrm{d}u} \cdot \frac{\mathrm{d}\vec{r}(u)}{\mathrm{d}u}}\, \mathrm{d}u. \tag{2.3}$$

Alternatively, by invoking the fundamental theorem of calculus, we also have the relation

$$\frac{\mathrm{d}s}{\mathrm{d}t} = \left|\left| \frac{\mathrm{d}\vec{r}(t)}{\mathrm{d}t} \right|\right|. \tag{2.4}$$

One reason why the arc length is an easy measure to work with, is that the tangent vector expressed in units of arc length becomes a unit vector. To see that this is true, we rewrite the expression (2.2) for the length of the curve in terms of the arc length:

$$L = \int_0^L \sqrt{\frac{\mathrm{d}\vec{r}(s)}{\mathrm{d}s} \cdot \frac{\mathrm{d}\vec{r}(s)}{\mathrm{d}s}}\, \mathrm{d}s. \tag{2.5}$$

Differentiating both sides of (2.5) with respect to L we find

$$\left|\left| \frac{\mathrm{d}\vec{r}(s)}{\mathrm{d}s} \right|\right| = 1. \tag{2.6}$$

The tangent vector is a useful enough quantity to give it its own symbol:

$$\hat{e}_s = \frac{\mathrm{d}\vec{r}(s)}{\mathrm{d}s}, \tag{2.7}$$

where we use the hat to indicate that $\hat{e}_s$ is a unit vector. By associating a tangent vector to every point of the curve we obtain a direction field on the curve. Intuitively it makes sense to associate the curvature of the curve with the rate of change of that direction field as we travel along the curve. A straight line then has zero curvature, whereas the curvature of a sharp bend is large. Splitting that rate of change in a magnitude and direction factor, we can write

$$\frac{\mathrm{d}\hat{e}_s}{\mathrm{d}s} = \kappa(s)\hat{n}(s), \tag{2.8}$$

where $\hat{n}$ is another unit vector. In fact, $\hat{n}(s)$ is perpendicular to $\hat{e}_s$, because the derivative of any unit vector $\hat{x}(s)$ is perpendicular to itself:

$$\begin{aligned} \frac{\mathrm{d}}{\mathrm{d}s}\left[\hat{x}(s)\cdot\hat{x}(s)\right] &= \frac{\mathrm{d}}{\mathrm{d}s}\left[1\right] \\ 2\hat{x}(s)\cdot\frac{\mathrm{d}\hat{x}(s)}{\mathrm{d}s} &= 0. \end{aligned} \tag{2.9}$$

The vector $\hat{n}(s)$ is called the unit normal of the curve and $\kappa(s)$ the curvature. By taking $\hat{n}(s)$ to be positive, the sign of $\kappa(s)$ tells us in which direction the curve is bent, whereas its magnitude tells us how sharp the bend is. Any energy functional we want to construct on the curve when relating it to a polymer should be independent of the direction in which we bend, and therefore can contain only even powers of κ. The most commonly used curvature energy is just the lowest (quadratic) power of κ integrated over the entire curve:

$$\mathcal{E}_{\text{curv}} = \frac{A}{2}\int_0^L \kappa(s)^2\, \mathrm{d}s. \tag{2.10}$$

Here A is a physical parameter, known as the bending modulus of the curve. Based on the energy (2.10) we can apply the toolbox of statistical physics on the ensemble of possible curves. Later on, we will develop a similar expression for the curvature energy of membranes.

Before we continue, there are two more observations to make about more intuitive definitions of the curvature. In colloquial talks and elementary courses the curvature is often defined as the inverse radius of the osculating circle at any point along the curve. That definition is completely equivalent to the one given here, although one loses the information stored in the sign of κ. To see that this is true, we express the magnitude of κ in terms of the original parametrization $\vec{r}(t)$:

$$|\kappa(t)| = \frac{||\vec{r}'(t) \times \vec{r}''(t)||}{||\vec{r}'(t)||^3}, \tag{2.11}$$

where primes denote derivatives with respect to t. If the osculating circle at $\vec{r}(t)$ has radius a, it is parametrized by $a(\cos t, \sin t)$. From equation (2.11), we immediately find that its curvature, and therefore that of the curve, is indeed $1/a$.

The other more intuitive definition is related to a quadratic expansion of the curve around a local minimum. Since our choice of coordinates of the embedding space $\mathbb{R}^2$ is arbitrary, we can always choose coordinates such that the origin is at the point of interest on the curve and that this point is also a local minimum in the coordinates chosen. Moreover, we can locally parametrize the curve by $\vec{r}(t) = (t, y(t))$. Since $\vec{r}(t)$ is a local minimum, the lowest order in the expansion of $y(t)$ is quadratic, and given by $\frac{1}{2}\kappa t^2$. The factor κ that multiplies the quadratic term is indeed the curvature as defined in equation (2.8), as is readily found by substituting the local expression for $\vec{r}(t)$ in equation (2.11) or alternatively equations (2.7) and (2.8). The interpretation of the curvature as the coefficient of the quadratic term in an expansion around a local minimum will be quite helpful later on when we consider the curvature of surfaces.

2.2.2 Curves in space

Curves in $\mathbb{R}^3$ enjoy an additional degree of freedom compared to their counterparts in $\mathbb{R}^2$. That means that at any point along the curve we now need three vectors as a basis for the space in which it lives, and that we can no longer describe the curve in that basis with a single parameter $\kappa(s)$. Instead we will need two parameters, the curvature $\kappa(s)$ (defined analogously to the two-dimensional case) and the torsion $\tau(s)$, which is related to the curve's chirality.

Like in two dimensions, we can parametrize a space curve using the arc length s and describe it in an arbitrary coordinate system by a vector $\vec{r}(s)$. The unit tangent vector $\hat{e}_s$ and normal $\hat{n}(s)$ now are three-dimensional vectors, but still defined by equations (2.7) and (2.8). The definition of the curvature $\kappa(s)$

is still given by equation (2.8) as well. Moreover, since the result (2.9) on the derivative of a unit vector holds in any number of dimensions, the unit tangent and unit normal vector are still orthonormal. To construct a basis for $\mathbb{R}^3$ at $\vec{r}(s)$ all we need to do is find a third vector which is perpendicular to both. That vector is given by their cross product and is known as the binormal

$$\hat{b}(s) = \hat{e}_s(s) \times \hat{n}(s). \tag{2.12}$$

Analogously to the definition of the curvature (2.8), we express the derivative of $\hat{n}(s)$ in terms of the basis $(\hat{e}_s, \hat{n}, \hat{b})$:

$$\frac{\mathrm{d}\hat{n}(s)}{\mathrm{d}s} = \alpha(s)\hat{e}_s + \tau(s)\hat{b}(s). \tag{2.13}$$

The quantity $\tau(s)$ is the torsion of the curve. The geometrical interpretation of the torsion is the rate of change of the osculating plane, the plane spanned by $\hat{e}_s$ and $\hat{n}$. The sign of the torsion is related to the curve's chirality: a left-handed curve has negative torsion, and the torsion of a right-handed curve is positive. The quantity $\alpha(s)$ in equation (2.16) is just the negative of $\kappa(s)$; to see that this is true we differentiate the relation $\hat{e}_s \cdot \hat{n} = 0$ expressing the orthogonality of $\hat{e}_s$ and $\hat{n}$:

$$0 = \frac{\mathrm{d}\hat{e}_s}{\mathrm{d}s} \cdot \hat{n} + \hat{e}_s \cdot \frac{\mathrm{d}\hat{n}}{\mathrm{d}s} = \kappa(s) + \alpha(s). \tag{2.14}$$

By also considering the derivative of $\hat{b}(s)$ we can find an easier expression for the torsion. We have

$$\begin{aligned} \frac{\mathrm{d}\hat{b}(s)}{\mathrm{d}s} &= \frac{\mathrm{d}\hat{e}_s}{\mathrm{d}s} \times \hat{n} + \hat{e}_s \times \frac{\mathrm{d}\hat{n}}{\mathrm{d}s} \\ &= \kappa\hat{n} \times \hat{n} + \hat{e}_s \times (-\kappa\hat{e}_s + \tau\hat{b}) \\ &= -\tau\hat{n}(s) \end{aligned} \tag{2.15}$$

so

$$\tau(s) = -\frac{\mathrm{d}\hat{b}(s)}{\mathrm{d}s} \cdot \hat{n}(s). \tag{2.16}$$

Like the curvature in the two-dimensional case, the combination of the curvature and the torsion at any point along the curve tells us the trajectory of the curve through space. That statement can be neatly summarized by combining the three-dimensional versions of equations (2.8), (2.13), and (2.15) into a single expression

$$\frac{\mathrm{d}}{\mathrm{d}s}\begin{pmatrix} \hat{e}_s(s) \\ \hat{n}(s) \\ \hat{b}(s) \end{pmatrix} = \begin{pmatrix} 0 & \kappa(s) & 0 \\ -\kappa(s) & 0 & \tau(s) \\ 0 & -\tau(s) & 0 \end{pmatrix} \begin{pmatrix} \hat{e}_s(s) \\ \hat{n}(s) \\ \hat{b}(s) \end{pmatrix}. \tag{2.17}$$

Equations (2.17) are known as the Frenet-Serret equations. They beautifully illustrate the symmetry between κ and τ: $\kappa(s)$ is the rate of rotation of $\hat{e}_s(s)$ around $\hat{b}(s)$ and $\tau(s)$ that of $\hat{n}(s)$ around $\hat{e}_s(s)$.

Much of the biophysical theory of polymers relies on the differential geometry of curves introduced in this section. Since our main focus is on membranes, those theories lie outside the scope of this text. For a further introduction see *e.g.* De Gennes [37] and Kamien [36].

2.3 Differential geometry of surfaces

2.3.1 Coordinate system and area element

Just like the curves in the previous section, a surface in three-dimensional space can be described in terms of the coordinates of that embedding space. Because the surface itself is two-dimensional, we will need two local coordinates to parametrize it. As was already alluded to in the introduction of this chapter, a particular choice of these coordinates may be valid only locally and not cover the entire surface, however, there will always a continuous bijection to another set of coordinates with which we can carry on. We will make use of this freedom of coordinate choice to choose a system best adapted to the particular problem at hand later on. For now we will take a set of two arbitrary coordinates (x_1, x_2) and write our mathematics in terms of them, making sure along the way that the results are independent of the particular choice we make here.

The first major difference with the curve is that on a surface there is no natural choice of coordinates like the arc length. Moreover, not only do we now need two numbers to characterize the curvature, there will actually be two ways of defining a proper coordinate independent curvature on the surface. One of them, the Gaussian curvature, will turn out to be intrinsic, which means it is not only independent of the coordinates chosen but also of the space in which we embed the surface. Moreover, the Gaussian curvature will be related to the topology of the surface. The other (extrinsic) curvature, known as the mean curvature, will play a role very similar to the curvature of the curve in the previous section.

Having chosen a coordinate system on the surface, we can associate a point in $\mathbb{R}^3$ with every point of the surface $\mathcal{M}$ and write

$$\mathcal{M} = \{\vec{r}(x_1, x_2) \,|\, x_1, x_2 \in \mathcal{U}\}, \tag{2.18}$$

where $\mathcal{U} \subset \mathbb{R}^2$ is the set of points over which x_1 and x_2 run. Similarly to the case of the curve, we can define tangent vectors to the surface by taking derivatives with respect to the parameters:

$$\vec{e}_1 = \frac{\partial \vec{r}(x_1, x_2)}{\partial x_1}, \tag{2.19}$$

$$\vec{e}_2 = \frac{\partial \vec{r}(x_1, x_2)}{\partial x_2}. \tag{2.20}$$

Lacking a natural length scale, we get tangent vectors which are neither necessarily normalized nor necessarily perpendicular to each other. Nonetheless, they do span a two-dimensional plane which is tangent to the surface at $\vec{r}(x_1, x_2)$. In order to construct a third vector which is perpendicular to both tangent vectors (so that the three of them span $\mathbb{R}^3$) we only need to calculate their cross product

$$\hat{n} = \frac{\vec{e}_1 \times \vec{e}_2}{||\vec{e}_1 \times \vec{e}_2||}, \tag{2.21}$$

where we have normalized this time to get a proper surface normal. By introducing the surface normal field on $\mathcal{M}$ (*i.e.*, by assigning a surface normal to each point of $\mathcal{M}$), we can classify the manifold as being *orientable* or *non-orientable*. The surface is orientable if at every point of the manifold we can consistently orient the tangent vectors $\vec{e}_1$ and $\vec{e}_2$ with respect to the normal $\hat{n}$, *e.g.* in such a way that using the right hand rule we can define a clockwise direction for every loop in the surface. For a surface which is both orientable and closed, we can use the normal vector field to define an inside and an outside of the manifold. Well-known examples of orientable, closed manifolds are the two-dimensional sphere and torus embedded in $\mathbb{R}^3$, and an example of a closed but non-orientable manifold is the Klein bottle. We will assume our manifolds to be closed and orientable from now on, and choose the direction of the normal vector such that it points outwards. We will also typically choose the coordinate system on $\mathbb{R}^3$ which we use to describe $\mathcal{M}$ such that its origin lies inside the space enclosed by the surface.

Using the tangent vectors defined above, we can calculate the infinitesimal area element at each point of the surface, and by integrating over $\mathcal{U}$ find the total surface area. The infinitesimal area element at $\vec{r}(x_1, x_2)$ is simply the area of the parallelogram spanned by the two tangent vectors, which in turn is given by the magnitude of their cross product:

$$\begin{aligned} \Delta S &= ||\vec{e}_1 \times \vec{e}_2|| \\ &= \sqrt{(\vec{e}_1 \times \vec{e}_2)^2} \\ &= \sqrt{||\vec{e}_1||^2||\vec{e}_2||^2 - (\vec{e}_1 \cdot \vec{e}_2)^2}. \end{aligned}$$

By putting back in the definitions of the tangent vectors we find the differential area element to be

$$\mathrm{d}S = \sqrt{(\partial_1\vec{r}(x_1,x_2))^2(\partial_2\vec{r}(x_1,x_2))^2 - (\partial_1\vec{r}(x_1,x_2)\cdot\partial_2\vec{r}(x_1,x_2))^2}\,\mathrm{d}x_1\,\mathrm{d}x_2, \tag{2.22}$$

where $\partial_i = \frac{\partial}{\partial x_i}$. The expression under the square root in equation (2.22) is exactly the determinant of the *induced metric* (or *first fundamental form*). The induced metric of an n-dimensional manifold with tangent vectors $\vec{e}_i$ is an (n, n) tensor given in component form by $g_{ij} = \vec{e}_i \cdot \vec{e}_j$; for our two-dimensional

manifold $\mathcal{M}$ it is given by

$$g(x_1,x_2) = \begin{pmatrix} \vec{e}_1(x_1,x_2)\cdot\vec{e}_1(x_1,x_2) & \vec{e}_1(x_1,x_2)\cdot\vec{e}_2(x_1,x_2) \\ \vec{e}_2(x_1,x_2)\cdot\vec{e}_1(x_1,x_2) & \vec{e}_2(x_1,x_2)\cdot\vec{e}_2(x_1,x_2) \end{pmatrix}. \tag{2.23}$$

For the total area of the manifold we can now write the elegant formula

$$\mathcal{A} = \int_{\mathcal{U}} \sqrt{\det g(x_1,x_2)}\,\mathrm{d}x_1\,\mathrm{d}x_2. \tag{2.24}$$

Although the expression (2.24) for $\mathcal{A}$ makes explicit use of a parametrization $\mathcal{U}$ of $\mathcal{M}$, the resulting area is independent of the parametrization chosen. To prove that statement, we consider a change of parametrization from a set of coordinates (x_1,x_2) that runs over $\mathcal{U}$ to another set (y_1,y_2) that runs over $\mathcal{V}$. Applying the chain rule, we find

$$\vec{e}_{x_i} = \frac{\partial\vec{r}}{\partial x_i} = \frac{\partial\vec{r}}{\partial y_k}\frac{\partial y_k}{\partial x_i} = \frac{\partial y_k}{\partial x_i}\vec{e}_{y_k}, \tag{2.25}$$

where we implicitly sum over the repeated index k. Applying the transformation (2.25) to the metric, we find

$$g_{ij}(x_1,x_2) = \frac{\partial y_k}{\partial x_i}\frac{\partial y_m}{\partial x_j}\tilde{g}_{km}(y_1,y_2), \tag{2.26}$$

where $\tilde{g}$ is the metric in the coordinate system (y_1,y_2). If we now define the transformation matrix X by $X_{ik} = \frac{\partial y_k}{\partial x_i}$, then we can rewrite equation (2.26) in matrix form as $g = X^T\tilde{g}X$. Returning to the expression (2.24) for the total membrane area, we find that a parameter transform does indeed not change the value of $\mathcal{A}$:

$$\begin{aligned} \mathcal{A} &= \int_{\mathcal{U}} \sqrt{\det g(x_1,x_2)}\,\mathrm{d}x_1\,\mathrm{d}x_2 \\ &= \int_{\mathcal{U}} \sqrt{\det(X^T\tilde{g}(x_1,x_2)X)}\,\mathrm{d}x_1\,\mathrm{d}x_2 \\ &= \int_{\mathcal{U}} \sqrt{\det\tilde{g}(x_1,x_2)}|\det X|\,\mathrm{d}x_1\,\mathrm{d}x_2 \\ &= \int_{\mathcal{V}} \sqrt{\det\tilde{g}(y_1,y_2)}\,\mathrm{d}y_1\,\mathrm{d}y_2, \end{aligned}$$

where the last equality holds because $|\det X|$ is exactly the Jacobian for the coordinate transformation from (x_1,x_2) to (y_1,y_2).

A parametrization that is often used is the *Monge gauge*, in which the membrane surface $\mathcal{S}$ is described as a height function $h(x,y)$ above $\mathbb{R}^2$ (parametrized by x and y). In that case we have $\vec{r} = (x,y,h(x,y))$ and the expression for

the total area reduces to

$$\mathcal{A} = \int_{\mathcal{U}} \sqrt{1 + \left(\frac{\partial h}{\partial x}\right)^2 + \left(\frac{\partial h}{\partial y}\right)^2}\, \mathrm{d}x\, \mathrm{d}y. \tag{2.27}$$

For objects such as soap films, which have no bending resistance, the only contribution to the total energy scales with the surface area

$$\mathcal{E}_{\text{area}} = \sigma \mathcal{A}, \tag{2.28}$$

where σ is the surface tension. A well-known example of a surface which minimizes the 'area energy' (2.28) is the shape of a soap film in between two rings, called a catenoid.

2.3.2 Curvature of surfaces

Even though biomembranes are fluid in their lateral direction and therefore, like the soap film, do not have any internal structure in that direction, their energy is not given by the simple expression (2.28). The membrane does have a characteristic bilayer structure in the direction normal to its surface, which means that bending the membrane will deform that structure and therefore carry an energy penalty. To construct a proper energy functional that describes the membrane shape we should therefore include curvature contributions.

As observed above, we will need two numbers at each point of the surface to characterize the curvature at that point. There is a straightforward way of getting two such numbers using the machinery we have already developed. Each of the combinations $(\vec{e}_1, \hat{n})$ and $(\vec{e}_2, \hat{n})$ of a tangent vector and the surface normal spans a plane which intersects $\mathcal{S}$ at our point of interest. The intersections are curves in $\mathbb{R}^2$, and the curvature of these curves in those planes are given by equation (2.8). Clearly these two curvatures of intersection lines depend on the particular choice of coordinates (x_1, x_2) we made. We get different values by rotating our coordinate axes, where any orientation (except parallel) of them with respect to each other is valid. By virtue of the surface being smooth these rotations will give us a maximum c_1 and minimum c_2 value of the intersection line curvatures. The numbers c_1 and c_2 are called the principal curvatures of the surface at (x_1, x_2), and their associated directions the principal directions (see figure 2.1a). By construction, the principal curvatures are independent of the choice of coordinates. They are however not the easiest quantities to work with. Instead, we use two combinations of them, known as the mean and Gaussian curvatures, which are defined as the average and product of the principal curvatures:

$$H = \frac{1}{2}(c_1 + c_2), \tag{2.29}$$

$$K = c_1 c_2. \tag{2.30}$$

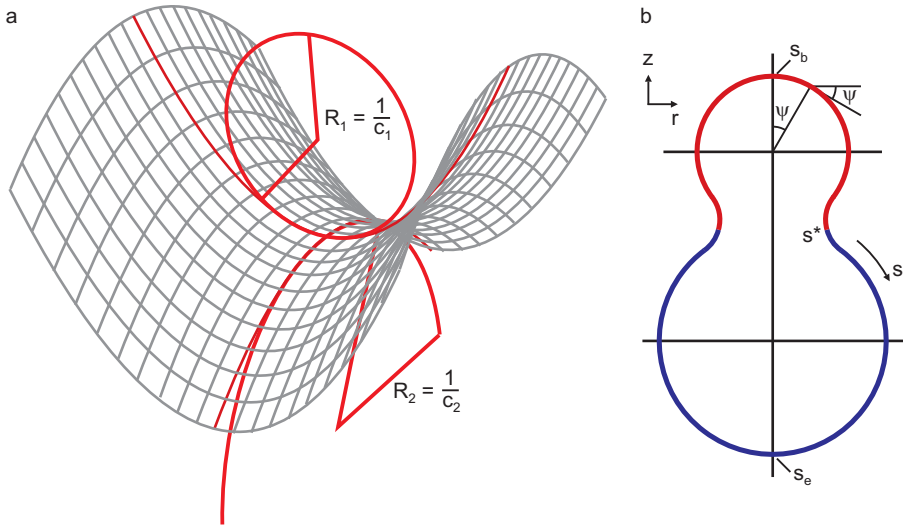

Figure 2.1: Curved surfaces. (a) A saddle point on a two-dimensional surface embedded in $\mathbb{R}^3$. The thick red lines indicate the principal directions. If the positive and negative curvatures are equal, the mean curvature at the saddle point is zero. If the surface extends to infinity, its Gaussian curvature is negative. (b) Coordinate system on an axisymmetric vesicle. The z-axis coincides with the axis of symmetry. The vesicle is parametrized using the arc length s along the contour. The radial coordinate r gives the distance from the symmetry axis and the coordinate z the distance along that axis. The shape of the vesicle be given as $r(z)$, $r(s)$, or in terms of the contact angle ψ of the contour as a function of either s or r. The geometric relations between r, z and ψ are given in equations (2.88) and (2.89).

Similar to the case of curves in $\mathbb{R}^2$, the principal curvatures c_1 and c_2 are the inverse of the radii of the osculating circles along their respective intersection curves. The definitions given in equations (2.29) and (2.30) are thus consistent with the intuitive, colloquial definitions of the previous section, but they are not easy to handle. Both in order to prove that H and K are indeed coordinate-independent, and for easier use in calculations involving the curvature energy later on, we will first formalize the definitions (2.29) and (2.30). In order to do that, we make use of the other, colloquial interpretation of curvature at the end of section 2.2.1. We choose a coordinate system on the embedding space $\mathbb{R}^3$ such that the origin is located at the point of interest $\vec{r}(x_1, x_2)$ and is a stationary point in the coordinates chosen. We can then express $\vec{r}(x_1, x_2)$ in the Monge gauge introduced at the end of section 2.3.1, and write $\vec{r}(x_1, x_2) = (x_1, x_2, z(x_1, x_2))$. Proceeding as before, we expand $z(x_1, x_2)$ around the minimum and find that the lowest-order term is quadratic in the coordinates:

$$z(x_1, x_2) - z_{\min} = \frac{1}{2}\vec{x}^T C \vec{x} + \text{h.o.t.} \tag{2.31}$$

where $\vec{x} = (x_1, x_2)^T$ and C is a symmetric matrix which is called the curvature matrix. Not surprisingly, we will find that c_1 and c_2 are the eigenvalues of C, and the corresponding eigenvectors are the principal directions.

Comparing equation (2.31) with the Taylor expansion of $z(x_1, x_2)$, we find for the coefficients of C ($i, j \in \{1, 2\}$):

$$C_{ij} = \frac{\partial^2 z(x_1, x_2)}{\partial x_i \partial x_j} = \frac{\partial^2 \vec{r}(x_1, x_2)}{\partial x_i \partial x_j} \cdot \hat{n}(x_1, x_2), \tag{2.32}$$

so the components of C are the projections of the second derivatives of $\vec{r}$ onto the surface normal $\hat{n}$. There are two (coordinate) invariants we can construct from the curvature matrix C: its trace and its determinant. They are directly related to the mean and Gaussian curvatures:

$$H = \frac{1}{2}\text{Tr}\, C = \frac{1}{2} g^{ij} C_{ij}, \tag{2.33}$$

$$K = \frac{\det C}{\det g}. \tag{2.34}$$

Here the g^{ij} are elements of the inverse of the metric tensor g and we once again sum over repeated indices.

It remains to show that the definitions (2.33) and (2.34) indeed are identical to the colloquial definitions (2.29) and (2.30) and that they are coordinate independent. To do so, we observe that since the matrix C is symmetric, it is diagonalizable by a orthonormal transformation T, $C = TDT^{-1}$, where $D = \text{diag}(d_1, d_2)$ with d_1 and d_2 the real eigenvalues of C. Moreover, if $d_1 \neq d_2$, then the corresponding eigenvectors are orthonormal, *i.e.*, they are unit vectors and perpendicular [38, Proposition 6.2]. If $d_1 = d_2$ then all directions are

principal directions, and we can choose any set of two orthonormal vectors that span the tangent plane. We denote these orthonormal vectors by $\hat{e}_1$ and $\hat{e}_2$ and, because D is just C expressed in the new basis $(\hat{e}_1, \hat{e}_2)$, we have

$$d_i = \big(\partial_i \hat{e}_i\big) \cdot \hat{n} \qquad (i = 1, 2), \tag{2.35}$$

where ∂_i as usual denotes the derivative along $\hat{e}_i$, and the unit vector $\hat{n}$ has not changed. In the new orthonormal basis, the metric is given by the identity matrix, so we find

$$H = \frac{1}{2}\big((\partial_1 \hat{e}_1) \cdot \hat{n} + (\partial_2 \hat{e}_2) \cdot \hat{n}\big). \tag{2.36}$$

Invoking equation (2.8) for the curvature of a line, this reduces to

$$H = \frac{1}{2}(\kappa_1 + \kappa_2) \tag{2.37}$$

with κ_i the curvature along $\hat{e}_i$. Since these were the principal directions, equation (2.37) is identical to equation (2.29).

There is an alternative expression for H in terms of the gradient of the surface normal, which immediately shows that it is coordinate-independent. Making use of the orthonormality of the basis $(\hat{e}_1, \hat{e}_2, \hat{n})$ and the Weingarten equations (2.55) derived in the next section, we can rewrite each of the terms of equation (2.36) in terms of derivatives of the unit normal:

$$(\partial_i \hat{e}_j) \cdot \hat{n} = \hat{e}_j \cdot -\partial_i \hat{n}. \tag{2.38}$$

For the mean curvature we then find:

$$H = -\frac{1}{2}\big(\hat{e}_1 \cdot \partial_1 \hat{n} + \hat{e}_2 \cdot \partial_2 \hat{n}\big) = -\frac{1}{2}\vec{\nabla} \cdot \hat{n}. \tag{2.39}$$

Equation (2.39) agrees with our intuitive understanding of curvature like equation (2.8) did: for a flat surface, the unit normal is constant and the mean curvature is zero. Once the surface gets bent, the unit normal changes and the absolute value of the mean curvature increases. Moreover, the expression given for H in equation (2.39) is indeed coordinate independent.

Relating the Gaussian curvature to the principle curvatures goes completely analogous to the mean curvature:

$$K = \frac{\det C}{\det g} = \frac{\det D}{1} = d_1 d_2 = \kappa_1 \kappa_2. \tag{2.40}$$

To show that the Gaussian curvature is coordinate independent, it is easiest to use the definition in terms of the ratio of determinants given by (2.34). Applying a coordinate transformation (2.25) which we again write in matrix form as X, we have $C = X^T \tilde{C} X$, $g = X^T \tilde{g} X$ and readily obtain:

$$K = \frac{\det C}{\det g} = \frac{\det(X^T \tilde{C} X)}{\det(X^T \tilde{g} X)} = \frac{\det X^T}{\det X^T} \frac{\det \tilde{C}}{\det \tilde{g}} \frac{\det X}{\det X} = \frac{\det \tilde{C}}{\det \tilde{g}}. \tag{2.41}$$

Alternatively, as we will see in section 2.3.4, the Gaussian curvature can be expressed as the inner product of the surface normal $\hat{n}$ with the curl of a vector field (equation (2.70), a form which is clearly coordinate independent.

2.3.3 Gauss's Theorema Egregium

The mean and Gaussian curvatures defined in the previous section are the invariants we will use to construct an energy functional for the membrane shape later on. To do so, there is no need to further develop the mathematical apparatus of surfaces. However, after we have defined that energy functional, we will make use of the Gauss-Bonnet theorem, which relates the integral of the Gaussian curvature to a topological boundary term, to simplify the expression significantly. In this section we will prove the earlier claim that the Gaussian curvature is an intrinsic property of the surface and in the next section we will derive the Gauss-Bonnet theorem. Before we can do that, we need to take a closer look at the metric and curvatures, and derive several useful identities. The proving technique for each of them is indicated here, but not always written out explicitly. For more details see *e.g.* Millman and Parker [33], Spivak [34] or Do Carmo [35].

In section 2.3.1, we defined the metric using the tangent vectors $\vec{e}_i$, which span the tangent plane $T_p\mathcal{M}$ to the point $p \in M$. We already used the metric to calculate the area of our manifold in equation (2.24), and here we will show that we can use it to calculate lengths and angles as well. Lines in the manifold have tangent vectors that lie in the tangent plane to the membrane at the point of interest. For an observer restricted to the manifold, components of vectors which lie along the manifold's surface normal $\hat{n}$ can not be measured, but components in the tangent plane can, because the manifold is locally flat. Quantities that can be expressed in terms of the tangent plane are therefore intrinsic to the manifold, the restricted observer can measure them without being aware of any embedding space. Due to the fact that the Gaussian curvature is intrinsic, this will allow the observer to determine that curvature from measurements that can be made within the manifold. To show that the earth is a sphere, it is therefore not necessary to go into space and take pictures from outside the manifold that is earth's surface; we could in principle prove this statement from ground measurements alone.

If we have a vector $\vec{v}$ tangent to $\mathcal{M}$ at p, we can express it in terms of the basis $(\vec{e}_1, \vec{e}_2)$ and write:

$$\vec{v} = v^i \vec{e}_i, \tag{2.42}$$

where once again we sum over repeated indices (which we continue to do throughout this chapter). The length of $\vec{v}$, and the angle θ between $\vec{v}$ and another vector $\vec{w} \in T_p\mathcal{M}$ can now be expressed in terms of the components of

the metric:

$$||\vec{v}||^2 = \vec{v}\cdot\vec{v} = v^i\vec{e}_i\cdot v^j\vec{e}_j = v^iv^jg_{ij} \tag{2.43}$$

$$||\vec{v}||\cdot||\vec{w}||\cos\theta = \vec{v}\cdot\vec{w} = v^iw^jg_{ij} \tag{2.44}$$

From measurements of lengths and angles of vectors within the manifold, we can determine the components of metric tensor g using equations (2.43) and (2.44). The metric is therefore an intrinsic property of the manifold, and any quantity that can be expressed in terms of the components of the metric is intrinsic as well.

In section 2.3.1 we introduced not only the metric, with components g_{ij}, but also its inverse, with components g^{ij}. The inverse metric has a geometrical interpretation of its own, due to the fact that there is an alternative way to define a basis for the tangent space $T_p\mathcal{M}$ at a point $p\in M$. We defined the basis vectors $\vec{e}_i$ as the derivatives of the manifold parametrization $\vec{r}(x_1,x_2)$ along the parameter x_i. We could equally well have taken the normals within $T_p\mathcal{M}$ to curves of constant x_i in the parametrization $\vec{r}(x_1,x_2)$ of $\mathcal{M}$. We choose the positive direction along that of increasing x_i, and denote these basis vectors by $\vec{e}^i$. By construction, we have

$$\vec{e}_1\cdot\vec{e}^2 = \vec{e}_2\cdot\vec{e}^1 = 0. \tag{2.45}$$

We now fix the length of the basis vectors $\vec{e}^i$ by imposing

$$\vec{e}_1\cdot\vec{e}^1 = \vec{e}_2\cdot\vec{e}^2 = 1. \tag{2.46}$$

Combining equations (2.45) and (2.46) we have $\vec{e}_i\cdot\vec{e}^j = \delta_i^j$. The metric with respect to the basis $(\vec{e}^1,\vec{e}^2)$ now has components $g^{ij} = \vec{e}^i\cdot\vec{e}^j$. To prove the claim that g^{ij} is the inverse of g_{ij}, we rewrite the vector $\vec{v}\in T_p\mathcal{M}$ of equation (2.42) in terms of the basis $(\vec{e}^1,\vec{e}^2)$:

$$\vec{v} = v_i\vec{e}^i. \tag{2.47}$$

The numbers v^i are called the contravariant components of $\vec{v}$ (with respect to the contravariant basis $(\vec{e}_1,\vec{e}_2)$) and the v_i are the covariant components (and $(\vec{e}^1,\vec{e}^2)$ the covariant basis). Analogously to (2.44) we can express the inner product of two vectors $\vec{v},\vec{w}\in T_p\mathcal{M}$ in terms of their covariant components and the covariant metric as $\vec{v}\cdot\vec{w} = g^{ij}v_iw_j$. Moreover, we can also mix the two bases and write

$$\vec{v}\cdot\vec{w} = v_i\vec{e}^i\cdot w^j\vec{e}_j = v_iw^j\delta_i^j = v_iw^j \tag{2.48}$$

so we now have four equivalent ways to write the inner product:

$$\vec{v}\cdot\vec{w} = g_{ij}v^iw^j = g^{ij}v_iw_j = v_iw^i = v^iw_i. \tag{2.49}$$

Equation (2.49) tells us that we can use g_{ij} and g^{ij} to translate between the two basis representations. Because $\vec{w}$ is arbitrary, we get from equality of respectively the second and fourth and third and fifth expressions in (2.49):

$$g_{ij}v^i = v_j \qquad \text{and} \qquad g^{ij}v_i = v^j \tag{2.50}$$

Colloquially we say that we can use the metric to raise and lower indices. Combining the two equalities in (2.50) we find for any vector $\vec{v} \in T_p\mathcal{M}$:

$$v_i = g_{ij}v^j = g_{ij}g^{jk}v_k \tag{2.51}$$

so by uniqueness of the representation of $\vec{v}$ in any basis

$$g_{ij}g^{jk} = \delta_i^k \tag{2.52}$$

and the metric of the contravariant and covariant representations are indeed each others inverse.

From the metric or first fundamental form, we now turn to the curvature matrix, which is also known as the second fundamental form or Weingarten map. Most differential geometry texts do not introduce it using the curvature of a paraboloid around a stationary point on the surface, but just define it using equation (2.32). This form is therefore a 2×2 matrix whose components are given by

$$L_{ij} = (\partial_i\vec{e}_j)\cdot\hat{n}, \tag{2.53}$$

where we follow convention and use the symbol L from now on. The components of the second fundamental form are thus the projections of the derivatives of the tangent vectors on the surface normals. Likewise, the Christoffel symbols are defined to be the projections on the surface tangents, and given by the equations

$$\partial_i\vec{e}_j = \Gamma_{ij}^k\vec{e}_k + L_{ij}\hat{n}. \tag{2.54}$$

Because $\hat{n}$ is a unit vector, we know that its derivative must be perpendicular to $\hat{n}$ (equation (2.9)). We can therefore write $\partial_i\hat{n}$ as a linear combination of the two tangent vectors. A straightforward calculation gives:

$$\partial_i\hat{n} = -L_{ij}g^{jk}\vec{e}_k. \tag{2.55}$$

Equations (2.55) are known as the Weingarten equations. We can use them to derive equation (2.38):

$$\begin{aligned}
\vec{e}_m\cdot\partial_i\hat{n} &= -L_{ij}g^{jk}\vec{e}_m\cdot\vec{e}_k \\
&= -L_{ij}g^{jk}g_{mk} \\
&= -L_{ij}\delta_m^j \\
&= -L_{im} \\
&= -(\partial_i\vec{e}_m)\cdot\hat{n}.
\end{aligned}$$

From equation (2.54) we can also find explicit expressions for the Christoffel symbols. By taking the dot product with $\vec{e}_l$ on both sides and subsequently multiplying with g^{lm} we find

$$\Gamma_{ij}^k = (\partial_i\vec{e}_j)\cdot\vec{e}_l g^{lk}. \tag{2.56}$$

Because $\partial_i \vec{e}_j = \frac{\partial^2 \vec{r}(x_1,x_2)}{\partial x_i \partial x_j} = \frac{\partial^2 \vec{r}(x_1,x_2)}{\partial x_j \partial x_i} = \partial_j \vec{e}_i$ we have $(\partial_i \vec{e}_j) \cdot \vec{e}_l = \frac{1}{2}\partial_i(\vec{e}_j \cdot \vec{e}_l)$ and by cyclically permutating indices in the last expression, we can rewrite Γ^k_{ij} as

$$\Gamma^k_{ij} = \frac{1}{2} g^{kl}(\partial_j g_{il} - \partial_l g_{ij} + \partial_i g_{lj}). \tag{2.57}$$

Equation (2.57) shows that the Christoffel symbols can be written in terms of the components of the metric tensors and its derivatives in the tangent plane. Hence the Christoffel symbols are intrinsic properties of the manifold.

Before we are ready to prove that the Gaussian curvature K is also intrinsic, we need one more mathematical object: the (Riemann) curvature tensor. It is defined in terms of the Christoffel symbols and thus reflects an intrinsic property of the manifold:

$$R^l_{ijk} = \partial_j \Gamma^l_{ik} - \partial_k \Gamma^l_{ij} + \Gamma^m_{ik}\Gamma^l_{mj} - \Gamma^n_{ij}\Gamma^l_{nk}. \tag{2.58}$$

Unlike the Christoffel symbols themselves, the Riemann tensor is an actual tensor, which means that under a change of coordinates it transforms as the four-parameter version of equation (2.25). We can express the Riemann curvature tensor in terms of the (extrinsic) components of the second fundamental form as

$$R^l_{ijk} = L_{ik}L_{jm}g^{ml} - L_{ij}L_{km}g^{ml}. \tag{2.59}$$

The 2^4 different equations expressed by (2.59) are known as Gauss's equations. The proof of (2.59) simultaneously provides us with another set of identities known as the Codazzi-Mainardi equations:

$$\partial_k L_{ij} - \partial_j L_{ik} = \Gamma^l_{ik}L_{jl} - \Gamma^l_{ij}L_{kl}. \tag{2.60}$$

The proof of equations (2.59) and (2.60) follows from the observation that

$$\partial_k(\partial_j \vec{e}_i) = \partial_j(\partial_k \vec{e}_i).$$

Expanding both sides using equations (2.54) and (2.55), we find that the tangential part of the resulting equality reproduces (2.59) and the normal component gives (2.60).

Gauss's equations allow us to express the Gaussian curvature

$$K = \det L / \det g$$

in terms of the Riemann curvature tensor. By equation (2.59) we have

$$g_{ln}R^l_{ijk} = (L_{ik}L_{jm}g^{ml} - L_{ij}L_{km}g^{ml})g_{ln} = L_{ik}L_{jn} - L_{ij}L_{kn}, \tag{2.61}$$

because $g^{ml}g_{ln} = \delta^m_n$. Now taking the special case that $i = k = 1, j = m = 2$, we find:

$$g_{l2}R^l_{121} = (L_{11}L_{22} - L_{12}L_{12}) = \det L = K \det g \tag{2.62}$$

so we can express K in terms of the intrinsic tensors R and g, which means that K itself is intrinsic. We have therefore proven what is known as Gauss's Theorema Egregium:

Theorem 2.1 (Theorema Egregium) *The Gaussian curvature K of a manifold $\mathcal{M}$ is an intrinsic property of that manifold.*

Theorem 2.1 tells us that we can measure the curvature of the manifold we live in without having to refer to a larger embedding space. That means we can establish the fact that the earth is an object with positive curvature without having to go to space - we could suffice with measuring the local metric coefficients. Similarly, the theory of general relativity uses this technique to determine the local curvature of the four-dimensional spacetime manifold on which the universe lives [39]. The fact that this is possible lead Gauss to label this theorem 'egregium', or remarkable. Originally, it was actually not this exact statement that Gauss called the theorema egregium, but an equivalent one, which relates the Gaussian curvature of two different surfaces if they are locally isometric.

Two two-dimensional manifolds (or surfaces) $\mathcal{M}$ and $\mathcal{N}$ are called isometric if there is an isometry between them. An isometry between $\mathcal{M}$ and $\mathcal{N}$ is a function $f : \mathcal{M} \to \mathcal{N}$ which is bijective, differentiable and preserves lengths, *i.e.*, for any curve $\gamma : [c,d] \subset \mathbb{R} \to \mathcal{M}$ the length of γ equals that of $f \circ \gamma$. The weaker condition that $\mathcal{M}$ and $\mathcal{N}$ are locally isometric is that for each point $p \in \mathcal{M}$ there exists an open subset $\mathcal{M}' \subset \mathcal{M}$ for which there is an isometry with an open subset $\mathcal{N}' \subset \mathcal{N}$. By considering the behavior of coordinate curves (curves obtained from a parametrization $\vec{r}(x_1, x_2)$ of $\mathcal{M}$ by keeping all except one of the coordinates fixed), it readily follows that if a local isometry exists, then the components of the metric in the open subsets $\mathcal{M}'$ and $\mathcal{N}'$ are identical (for a written out version of the proof of that statement, see [33, Proposition 10.5]). Because by Theorem 2.1 the Gaussian curvature K is completely determined by the components of the metric, we have the following corollary:

Corollary 2.2 *If two surfaces are locally isometric, then their Gaussian curvatures at corresponding points are equal.*

2.3.4 The Gauss-Bonnet Theorem

The Theorema Egregium tells us that the Gaussian curvature can be measured using only the intrinsic properties of the surface it is defined on. The Gauss-Bonnet theorem will give us an easy method to do just that. Moreover, it will relate two properties of the surface which do not seem to have any connection at all: its geometry and its topology. In fact, we will find that the integrated Gaussian curvature over a closed surface is a constant dependent only on the genus of the surface, and that the Gaussian curvature of a patch of surface is related to the in-surface (or geodesic) curvature of its boundary. Boundaries of patches of surfaces are curves in the embedding space $\mathbb{R}^3$, which we have already studied in section 2.2.2. For a curve constrained to a surface we can of

course use the properties of both, and will indeed do so. To avoid confusion, we need to distinguish between the basis vectors defined using the surface and those defined using the curve. We will keep the notation of this section and denote the basis vectors of the surface by $(\vec{e}_1, \vec{e}_2, \hat{n})$. The tangent, normal and binormal vectors defined on the three-dimensional space curve we will denote using capital letters: $(\hat{T}(s) = \hat{e}_s, \hat{N}(s), \hat{B}(s))$, where s is the arc length along the curve. By construction $\hat{T}$ is tangent to both the curve and the surface, but in general $\hat{N}$ and $\hat{B}$ have components both tangent and normal to the surface.

For simplicity we make a change of basis from $(\vec{e}_1, \vec{e}_2, \hat{n})$ to an orthonormal system, for example by taking by taking $\hat{e}_1 = \vec{e}_1/||\vec{e}_1||$ and $\hat{e}_2 = \frac{\vec{e}_2 - (\vec{e}_2 \cdot \hat{e}_1)\hat{e}_1}{||\vec{e}_2 - (\vec{e}_2 \cdot \hat{e}_1)\hat{e}_1||}$. We consider a curve γ on the surface $\mathcal{M} \subset \mathbb{R}^3$, and denote these basis vectors at the point $\gamma(s) = \vec{r}(x_1, x_2) \in \mathcal{M}$ by $(\hat{e}_1(s), \hat{e}_2(s), \hat{n}(s))$. Because the tangent vector $\hat{T}(s)$ to γ is tangent to $\mathcal{M}$ as well, we can write

$$\hat{T}(s) = \cos(\theta(s))\hat{e}_1(s) + \sin(\theta(s))\hat{e}_2(s). \tag{2.63}$$

As we travel along γ, the basis $(\hat{e}_1(s), \hat{e}_2(s), \hat{n}(s))$ changes orientation in space, and γ itself may change orientation within $\mathcal{M}$. Both effects are accounted for in equation (2.63), but it will be useful to separate the two. To do so, we consider a vector field $\vec{P}(s)$ defined on γ with the conditions that $\vec{P}(s)$ lies in the plane spanned by $(\hat{e}_1, \hat{e}_2)$ and all vectors $\vec{P}$ are parallel in the embedding space $\mathbb{R}^3$, or $d\vec{P}/ds = 0$. By expressing $\vec{P}$ in terms of $(\hat{e}_1, \hat{e}_2)$ like in equation (2.63), we will be able to determine the effect of the change of orientation of the basis alone. However, we first need to verify that such a vector field $\vec{P}$ indeed exists. A straightforward expansion of the condition $\frac{\mathrm{d}\vec{P}}{\mathrm{d}s} \cdot \hat{e}_j = 0$ in contravariant components P^k of $\vec{P}$ shows that they satisfy the coupled differential equations

$$\frac{\mathrm{d}P^k}{\mathrm{d}s} = -\Gamma^k_{ij} P^i \frac{\mathrm{d}\gamma^j}{\mathrm{d}s}. \tag{2.64}$$

By the Picard-Lindelöf Theorem (see *e.g.* [40]), the system of ordinary differential equations (2.64) has a unique solution for a given initial condition $\vec{P}(s = 0) = \vec{P}_0$, so the vector field we need does indeed exist. Using the fact that $s = s(\vec{x}) = s(x_1, x_2)$ and expressing $\vec{P}$ in the basis $(\hat{e}_1, \hat{e}_2)$, we have

$$\vec{P}(\vec{x}) = \cos(\theta_0(\vec{x}))\hat{e}_1(\vec{x}) + \sin(\theta_0(\vec{x}))\hat{e}_2(\vec{x}). \tag{2.65}$$

Taking derivatives of $\vec{P}$ along $\hat{e}_1$ and $\hat{e}_2$, we can relate variations of the basis to variations of θ_0:

$$\begin{aligned} 0 = \hat{e}_1(\vec{x}) \cdot \partial_i \vec{P}(\vec{x}) &= -\sin(\theta_0(\vec{x}))\big(\partial_i \theta_0(\vec{x}) - \hat{e}_1(\vec{x}) \cdot \partial_i \hat{e}_2(\vec{x})\big) && (2.66) \\ 0 = \hat{e}_2(\vec{x}) \cdot \partial_i \vec{P}(\vec{x}) &= \cos(\theta_0(\vec{x}))\big(\partial_i \theta_0(\vec{x}) + \hat{e}_2(\vec{x}) \cdot \partial_i \hat{e}_1(\vec{x})\big) \\ &= \cos(\theta_0(\vec{x}))\big(\partial_i \theta_0(\vec{x}) - \hat{e}_1(\vec{x}) \cdot \partial_i \hat{e}_2(\vec{x})\big) && (2.67) \end{aligned}$$

where we used the orthogonality of $\hat{e}_1$ and $\hat{e}_2$ in the final equality. We can combine equations (2.66) and (2.67) in a single expression:

$$\vec{\nabla}\theta_0(\vec{x}) = \hat{e}_1(\vec{x}) \cdot \vec{\nabla}\hat{e}_2(\vec{x}) \equiv \vec{\Omega}(\vec{x}), \tag{2.68}$$

where the vector field $\vec{\Omega}$ is known as the spin connection. Equation (2.68) tells us how the basis $(\hat{e}_1(s), \hat{e}_2(s))$ changes as we move along γ; to find the change of $\hat{T}$ due to changes in orientation of γ, we should look at the gradient of $\theta(\vec{x}) - \theta_0(\vec{x})$. The 'true change' in $\hat{T}$ is therefore given by the covariant derivative of $\theta(\vec{x})$:

$$\vec{D}\theta(\vec{x}) \equiv \vec{\nabla}\theta(x) - \vec{\Omega}(\vec{x}). \tag{2.69}$$

The spin connection $\vec{\Omega}$ is defined using gradients of the basis vectors $\hat{e}_i$. We encountered those before, in the definition of the Gaussian curvature K, using the determinant of the second fundamental form L. The components of that form were the projections of the derivatives of the basis vectors $\vec{e}_i$ on the surface normal $\hat{n}$. Not surprisingly, the spin connection and Gaussian curvature are related. Expanding the curl of $\vec{\Omega}$ and the determinant of L in components of the basis $(\hat{e}_1, \hat{e}_2, \hat{n})$, we readily obtain the identity [36]

$$K = \hat{n} \cdot (\vec{\nabla} \times \vec{\Omega}). \tag{2.70}$$

We are now ready to face the task set at the beginning of this section: the calculation of the integral of the Gaussian curvature over a surface patch $\mathcal{M}$ with boundary $\gamma = \partial\mathcal{M}$. As observed before, the tangent vector $\hat{T}(s)$ to γ is also tangent to $\mathcal{M}$, but the curve normal $\hat{N}(s)$ is not necessarily tangent to $\mathcal{M}$ as well. An observer living on the surface $\mathcal{M}$ can therefore not measure the curvature $\kappa(s)$ of γ, since by equation (2.8) that requires knowledge of the component of $\hat{N}$ normal to $\mathcal{M}$. However, the component of the curvature of γ in $\mathcal{M}$ can be measured. This component is known as the geodesic curvature[1] and is given by the projection of $\hat{T}'(s)$ on the tangent plane of $\mathcal{M}$:

$$\begin{aligned} \kappa_g(s) &= \hat{T}'(s) \cdot \big(\hat{n}(s) \times \hat{T}(s)\big) \\ &= \hat{n}(s) \cdot \big(\hat{T}(s) \times \hat{T}'(s)\big) \\ &= \partial_s\theta(s) - \hat{e}_1(s) \cdot \partial_s\hat{e}_2(s) \end{aligned} \tag{2.71}$$

where we expressed $\hat{T}$ in terms of the basis $(\hat{e}_1, \hat{e}_2)$ using (2.63) again. Rewriting equation (2.71) in terms of the parametrization (x_1, x_2), we find that we can express the geodesic curvature as the projection of the covariant derivative of θ on the tangent $\hat{T}$:

$$\kappa_g(s(\vec{x})) = \big(\vec{D}(\theta(\vec{x}))\big) \cdot \hat{T}(\vec{x}). \tag{2.72}$$

[1] The projection of $\hat{T}'(s)$ on $\hat{n}(s)$ is known as the normal curvature $\kappa_n(s)$, and the total curvature satisfies $\kappa^2 = \kappa_g^2 + \kappa_n^2$.

Using Stokes' Theorem to relate the surface integral over the curl of $\vec{\Omega}$ to the line integral over the surface boundary of Ω, we have:

$$\int_{\mathcal{M}} \left(\vec{\nabla} \times \vec{\Omega}(\vec{x})\right) \cdot \mathrm{d}\vec{S} = \oint_{\partial\mathcal{M}} \vec{\Omega}(\vec{x}) \cdot \mathrm{d}\vec{r}, \tag{2.73}$$

where $\mathrm{d}\vec{S} = \hat{n}\,\mathrm{d}S$ and $\mathrm{d}\vec{r} = \hat{T}\,\mathrm{d}s$. The surface integral over the Gaussian curvature K and the line integral over the geodesic curvature κ_g thus add up to a simple expression:

$$\begin{aligned} \int_{\mathcal{M}} K\,\mathrm{d}S + \oint_{\partial\mathcal{M}} \kappa_g(s)\,\mathrm{d}s &= \int_{\mathcal{M}} \vec{\nabla} \times \vec{\Omega}(\vec{x}) \cdot \mathrm{d}\vec{S} \\ &\qquad + \oint_{\partial\mathcal{M}} \left(\vec{\nabla}\theta(\vec{x}) - \vec{\Omega}(\vec{x})\right) \cdot \mathrm{d}\vec{r} \\ &= \oint_{\partial\mathcal{M}} \frac{\mathrm{d}\theta(s)}{\mathrm{d}s}\,\mathrm{d}s. \end{aligned} \tag{2.74}$$

If the boundary curve is smooth and does not intersect itself, it makes a single closed loop, and the tangent vector $\hat{T}$ rotates around the surface normal $\hat{n}$ exactly once, so the integral over $d\theta/ds$ equals 2π. There could be kinks in the boundary curve $\gamma = \partial\mathcal{M}$, in which case we get $2\pi - \sum_i(\pi - \Delta\theta_i)$, with $\Delta\theta_i$ the interior angle of the i^{th} kink. Equation (2.74) is known as the Gauss-Bonnet formula. It allows us to calculate the integral over K for a closed surface of any genus (*i.e.*, with any number of holes), by cutting it up into regular patches for which equation (2.74) holds. Using such a decomposition, it readily follows that for any region $\mathcal{R}$ on an oriented surface $\mathcal{M}$ the following theorem is true.

Theorem 2.3 (Gauss-Bonnet) *Let $\mathcal{R}$ be a region on an oriented surface $\mathcal{M} \subset \mathbb{R}^3$ with piecewise continuous boundary γ. Then*

$$\int_{\mathcal{R}} K\,\mathrm{d}S + \oint_{\gamma} \kappa_g\,\mathrm{d}s + \sum_i (\pi - \Delta\theta_i) = 2\pi\chi(\mathcal{R}), \tag{2.75}$$

where the $\Delta\theta_i$ are the interior angles of γ and $\chi(\mathcal{R})$ is the Euler characteristic of $\mathcal{R}$. In particular, for a closed compact surface $\mathcal{M}$ of genus g we have

$$\int_{\mathcal{M}} K\,\mathrm{d}S = 2\pi\chi = 4\pi(1-g). \tag{2.76}$$

The proof of Theorem 2.3 sketched here is from Kamien [36]. An alternative proof using geodesic coordinate patches can be found in Millman and Parker [33].

2.3.5 The Canham-Helfrich free energy functional

In this final section we return to the biological membrane and apply the results of this chapter to find a mathematical description for them. We derive

an expression for the energy of a membrane and evaluate it for a few special cases. We also give the general shape equation for a uniform membrane. For nonuniform membranes, we apply the formalism to find both the equations for uniform domains as well as their boundary conditions.

The Canham-Helfrich free energy functional describes the contribution to the total free energy of a membrane due to the curvature of that membrane. A special case was introduced by Canham in 1970 when studying the biconcave shape of red blood cells [41]. The general expression was given by Helfrich in 1973 [42]. Of course the curvature energy must be coordinate invariant, which means it must be expressed in terms of the principal curvatures introduced in section 2.3.2, or equivalently in terms of the mean H and Gaussian K curvatures. The Canham-Helfrich curvature energy contains all possible linear and quadratic terms in the principal curvatures, and is given by

$$\mathcal{E}_{\text{curv}} = \int_{\mathcal{M}} \left(\frac{\kappa}{2}(2H - C_0)^2 + \bar{\kappa}K \right) \mathrm{d}S. \tag{2.77}$$

Here the physical parameters κ and $\bar{\kappa}$ are the bending and Gaussian moduli respectively. For a biological or biomimetic membrane consisting of various types of lipids, they can be uniform throughout the membrane if the lipids are well mixed, but they can also vary if the lipids separate into domains. The parameter C_0 is the *spontaneous curvature*. The only term in (2.77) which is linear in the principle curvatures scales with C_0. The spontaneous curvature reflects the possibility of an asymmetry between the two leaflets of the membrane. For $C_0 = 0$, all terms in (2.77) are quadratic in the principal curvatures and the energy of a membrane patch is symmetric under reflections. Putting $C_0 \neq 0$ breaks this symmetry. We assume the spontaneous curvature to vanish in our description of the experiments involving phase separation in biomimetic vesicles, because there is no reason to assume an asymmetry between the leaflets is introduced when making these vesicles by means of electroformation (see appendix 4.A and [43] for details on the experimental procedures). Moreover, in the experiments we use the membrane leaflets have had ample time to relax any asymmetries that may still have formed by flipping lipids from one leaflet to the other. Finally, the descriptions of the membrane shapes we obtain with $C_0 = 0$ give accurate fits to the experimental data, confirming that assuming the spontaneous curvature to vanish in this case is justified. In contrast, when the membrane contains proteins which have a nonsymmetric (typically cone-like) shape, spontaneous curvature plays an important role and should be included.

For vanishing C_0, the Canham-Helfrich curvature energy (2.77) takes on the simple form

$$\mathcal{E}_{\text{curv}} = \int_{\mathcal{M}} \left(\frac{\kappa}{2}(2H)^2 + \bar{\kappa}K \right) \mathrm{d}S. \tag{2.78}$$

In the case of a uniform and closed membrane without holes (*i.e.*, with the topology of a sphere), like that of a red blood cell, the Gauss-Bonnet theorem

tells us that the integral over K is a constant, which we can simply subtract from the total energy. In many papers, including the original one by Canham, this term is therefore left out. For a membrane with piecewise constant composition (*i.e.*, with patches in which the physical parameters are uniform), the Gauss-Bonnet theorem tells us that the integral of K over such a uniform patch of membrane is related to a boundary term. Within the patch the only contribution to the curvature energy is therefore given by the mean curvature H.

Of course a membrane still has an area energy (2.28) like the soap films in section 2.3.1 did. We can consider the area energy from two viewpoints: either we take the total area of the membrane to be fixed (in which case we have a constraint on the shape, and the area energy $\mathcal{E}_{\text{area}}$ is constant) or we use the surface tension σ as a Lagrange multiplier for the membrane area $\mathcal{A}$. In the latter case, the total energy of a closed, single-component membrane without holes can be written as

$$\mathcal{E} = \mathcal{E}_{\text{curv}} + \mathcal{E}_{\text{area}} = \int_{\mathcal{M}} \left(\frac{\kappa}{2}(2H)^2 + \sigma \right) \mathrm{d}S, \tag{2.79}$$

where we have left out the constant contribution of the Gaussian curvature. The shape that minimizes (2.79) for a given membrane surface area $\mathcal{A}$ is the one that minimizes the overall mean curvature. It is a straightforward result that that shape is the most regular one possible, namely the sphere of radius $R = \sqrt{\mathcal{A}/4\pi}$. Interestingly, the curvature energy of such a sphere is independent of its radius:

$$\mathcal{E}_{\text{curv}} = \frac{\kappa}{2} \int_{\mathcal{M}} (2H)^2 \, \mathrm{d}S = \frac{\kappa}{2} \int_{\mathcal{M}} \left(\frac{1}{R} + \frac{1}{R} \right)^2 R^2 \, \mathrm{d}\Omega = 8\pi\kappa. \tag{2.80}$$

To get more interesting shapes, we should apply additional conditions. One such condition is to actively perturb the membrane by exerting a point force on a large spherical membrane vesicle. Experimental results show that applying such a force on a 'giant' unilamellar vesicle (or GUV, with a radius of $10-50\ \mu\mathrm{m}$) results in the extraction of a cylindrical membrane tube with uniform cross section [45]. In this case the total energy of the system is given by

$$\mathcal{E} = \int_{\mathcal{M}} \left(\frac{\kappa}{2}(2H)^2 + \sigma \right) \mathrm{d}S - fL, \tag{2.81}$$

where f is the applied force and L is the displacement of the point where the force is attached in the direction of that force. Specifically, for a cylindrical tube of radius R and length L equation (2.81) reads

$$\mathcal{E}_{\text{tube}} = \left(\frac{\kappa}{2} \frac{1}{R^2} + \sigma \right) 2\pi RL - fL. \tag{2.82}$$

Equation (2.82) shows a competition between two effects: the bending rigidity term tries to increase the tube radius, whereas the surface tension term tries to

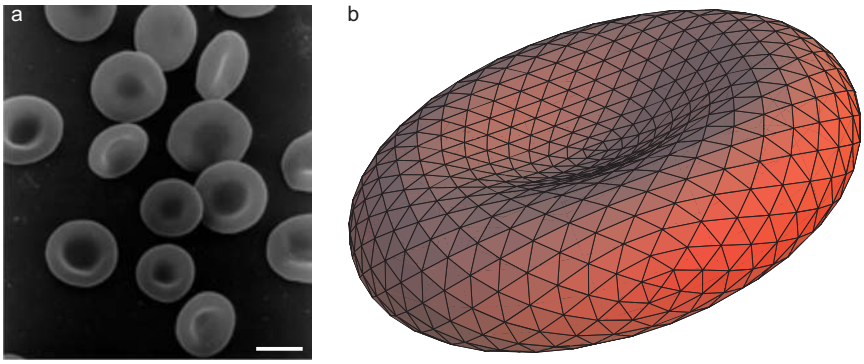

Figure 2.2: Shape of red blood cells. (a) Micrograph of human red blood cells, showing their distinct biconcave shape. Image courtesy of the National Institutes of Health (U.S.A.), scalebar $5\ \mu\mathrm{m}$. (b) Numerically obtained shape of a red blood cell, from the minimization of the bending energy (2.78), for a fixed enclosed volume and membrane area. The calculations were performed using the Surface Evolver software package by Brakke [44].

reduce it. A stable solution for an applied force f_0 can be obtained by choosing the proper radius R_0 such that the two effects exactly cancel. The values of f_0 and R_0 for given κ and σ are found from the stability condition that the derivatives of $\mathcal{E}_{\text{tube}}$ with respect to R and L should vanish. They give [46, 47]:

$$R_0 = \sqrt{\frac{\kappa}{2\sigma}}, \tag{2.83}$$

$$f_0 = 2\pi\sqrt{2\kappa\sigma}. \tag{2.84}$$

For typical values of $\kappa \approx 40\ \mathrm{pN\ nm}$ and $\sigma = 0.05\ \mathrm{pN/nm}$ we get $R_0 \approx 20\ \mathrm{nm}$ and $f_0 \approx 13\ \mathrm{pN}$. The tube radius is thus several orders of magnitude smaller than that of an experimental vesicle, which means that the implicit assumptions that any surface and volume constraints on the tube could be ignored, were justified. In chapter 7 we study such tubes as they are extracted not by an experimentally applied force, but by molecular motors.

An alternative additional condition is to fix the volume enclosed by the membrane. The sphere is the shape that encloses the maximal volume given its area; by forcing the volume to be less than that of a sphere we therefore create some 'excess area'. One particular such shape is the biconcave one of the red blood cell, where the enclosed volume is about half that of the sphere with the same area. Analytical expressions for such shapes are not easy to obtain, but numerically minimizing the curvature energy of a uniform closed membrane given an enclosed volume and total membrane area is a tractable task.

The software package Surface Evolver by Brakke [44] does just that. Figure 2.2 shows an example numerical result, where we begin with an arbitrary shape with the set amount of enclosed volume and surface area, and allow the curvature energy to relax. In our numerical calculations, independent of the original shape, we invariably retrieved the biconcave shape of the red blood cell.

In general, a differential equation for the mean curvature of a closed uniform vesicle with specified area and enclosed volume can be obtained through variation analysis. The energy is in this case given by the (mean) curvature energy with two Lagrange multiplier terms, one for the area (where the multiplier is the surface tension) and one for the volume (where the multiplier is the pressure difference across the membrane):

$$\mathcal{E} = \int_{\mathcal{M}} \left(\frac{\kappa}{2}(2H)^2 + \sigma \right) \mathrm{d}S + p \int \mathrm{d}V \tag{2.85}$$

The calculation of the first variation of this energy is lengthy but straightforward and was first performed by Ou-Yang and Helfrich [48]. The condition that this variation should vanish for an equilibrium shape results in the shape equation

$$p - 2\sigma H + 4\kappa H(H^2 - K) + 2\kappa \Delta H = 0, \tag{2.86}$$

where

$$\Delta = \frac{1}{\sqrt{\det g}} \partial_i \left(g^{ij} \sqrt{\det g} \partial_j \right) \tag{2.87}$$

is the Laplace-Beltrami differential operator on the membrane surface $\mathcal{M}$.

Equation (2.86) becomes a lot more tractable if we apply it to axisymmetric vesicles. Such vesicles are completely specified by giving the contour shape in a plane which contains the axis of rotation. Typically the axes of this plane are labelled r (horizontal) and z (vertical), where the z-axis is the axis of rotation. Because the contour is a curve in $\mathbb{R}^2$ we can parametrize it using the arc length along the contour from an arbitrary starting point, typically the topmost point of the contour. The coordinates $r(s)$ and $z(s)$ of any point on the contour are then related via the contact angle $\psi(s)$ on any point of the contour (see figure 2.1b):

$$\dot{r} = \frac{\mathrm{d}r}{\mathrm{d}s} = \cos\psi(s), \tag{2.88}$$

$$\dot{z} = \frac{\mathrm{d}z}{\mathrm{d}s} = -\sin\psi(s). \tag{2.89}$$

We can also express the mean and Gaussian curvatures and the Laplace-Bel-

trami operator in terms of $\psi(s)$ and $r(s)$:

$$H = -\frac{1}{2}\left(\dot{\psi} + \frac{\sin\psi(s)}{r(s)}\right) \tag{2.90}$$

$$K = \frac{\sin\psi(s)}{r(s)}\dot{\psi} \tag{2.91}$$

$$\Delta = \frac{\partial^2}{\partial s^2} + \frac{\dot{r}}{r}\frac{\partial}{\partial s} + \frac{1}{r^2}\frac{\partial^2}{\partial \phi^2} - \frac{\dot{r}}{r^3}\frac{\partial}{\partial \phi} \tag{2.92}$$

where ϕ is the polar angle, which runs from 0 to 2π. Substituting the axisymmetric expressions in the shape equation (2.86) we obtain the third-order differential equation for $\psi(s)$ [49]:

$$\begin{aligned}\dddot{\psi} = &-\frac{2\cos\psi}{r}\ddot{\psi} - \frac{1}{2}\dot{\psi}^3 + \frac{3\sin\psi}{2r}\dot{\psi}^2 + \frac{3\cos^2\psi - 1}{2r^2}\dot{\psi} \\ &+\frac{\sigma}{\kappa}\dot{\psi} - \frac{\cos^2\psi + 1}{2r^3}\sin\psi + \frac{\sigma}{\kappa}\frac{\sin\psi}{r} - \frac{p}{\kappa}.\end{aligned} \tag{2.93}$$

As was shown by Zheng and Liu [50], equation (2.93) can be written as a total derivative, which can be integrated to give an equivalent second order differential equation for $\psi(s)$:

$$\begin{aligned}\ddot{\psi}\cos\psi = &-\frac{1}{2}\sin\psi\dot{\psi}^2 - \frac{\cos^2\psi}{r}\dot{\psi} \\ &+\frac{\cos^2\psi + 1}{2r^2}\sin\psi + \frac{\sigma}{\kappa}\sin\psi - \frac{p}{\kappa}r.\end{aligned} \tag{2.94}$$

There is an alternative way of deriving the differential equations (2.93) and (2.94), by writing the energy (2.85) as an action, or an integral over a Lagrangian $\mathcal{L} = \mathcal{L}(\psi, \dot{\psi}, r, \dot{r}, z, \dot{z})$. This approach has the advantage that it gives us the proper differential equation for each axisymmetric patch of the vesicle surface, and also the conditions at their boundaries [51, 52]. For a patch that runs from $s = s_1$ to $s = s_2$ we have

$$\mathcal{E} = 2\pi\kappa\int_{s_1}^{s_2}\mathcal{L}\,\mathrm{d}s, \tag{2.95}$$

with

$$\begin{aligned}\mathcal{L} = &\frac{r}{2}\left(\dot{\psi} + \frac{\sin\psi}{r}\right)^2 + \frac{\sigma}{\kappa}r + \frac{p}{2\kappa}r^2\sin\psi \\ &+\gamma(\dot{r} - \cos\psi) + \eta(\dot{z} + \sin\psi).\end{aligned} \tag{2.96}$$

In equation (2.96) we used (2.90) to express H in terms of ψ and added two additional Lagrange multipliers γ and η to enforce the geometrical relations

(2.88) and (2.89). Variation of the functional $\mathcal{E}$ with respect to the variables ψ, r, z, γ and η gives their respective Euler-Lagrange equations, which for any variable x read

$$\frac{\mathrm{d}}{\mathrm{d}s}\frac{\partial \mathcal{L}}{\partial \dot{x}} - \frac{\partial \mathcal{L}}{\partial x} = 0. \tag{2.97}$$

From the variations with respect to the Lagrange multipliers γ and η we recover (2.88) and (2.89). The other three Euler-Lagrange equations give the following equations for the bulk of the patch:

$$\begin{aligned}
\ddot{\psi} &= \frac{\cos\psi\sin\psi}{r^2} - \frac{\cos\psi}{r}\dot{\psi} + \frac{p}{2\kappa}r\cos\psi \\
&\quad \frac{\gamma}{r}\sin\psi + \frac{\eta}{r}\cos\psi, && (2.98)\\
\dot{\gamma} &= \frac{1}{2}\dot{\psi}^2 - \frac{\sin^2\psi}{2r^2} + \frac{\sigma}{\kappa} + \frac{p}{\kappa}r\sin\psi, && (2.99)\\
\dot{\eta} &= 0. && (2.100)
\end{aligned}$$

There is an additional constraint which has to be taken into account, namely that the variation of $\mathcal{E}$ with respect to variations in the contour length, or equivalently the endpoints s_1 and s_2, should vanish. This condition is accounted for by demanding that the Hamiltonian $\mathcal{H}$ (defined below) should satisfy $\mathcal{H}(s_1) = \mathcal{H}(s_2) = 0$. Because the Lagrangian $\mathcal{L}$ does not depend directly on the arc length s, this implies that $\mathcal{H}$ should vanish everywhere. We therefore get an additional equation:

$$\begin{aligned}
\mathcal{H} &\equiv -\mathcal{L} + \dot{\psi}\frac{\partial \mathcal{L}}{\partial \dot{\psi}} + \dot{r}\frac{\partial \mathcal{L}}{\partial \dot{r}} + \dot{z}\frac{\partial \mathcal{L}}{\partial \dot{z}} \\
&= \frac{r}{2}\left[\dot{\psi}^2 - \left(\frac{\sin\psi}{r}\right)^2\right] - \frac{\sigma}{\kappa}r - \frac{p}{2\kappa}r^2\sin\psi && (2.101)\\
&\quad +\gamma\cos\psi - \eta\sin\psi \\
&= 0.
\end{aligned}$$

We can combine equations (2.98, 2.99, 2.100) and (2.101) to reproduce equation (2.93). First we rewrite (2.101) to obtain η, which we substitute in (2.98) to get an expression for γ in terms of ψ. Differentiating that expression with respect to s and relating it to (2.99) we find (2.93).

A large part of this thesis is dedicated to vesicles with multiple domains. For such a vesicle, the energy given by equation (2.85) is incorrect, since it ignores the Gaussian curvature, which by the Gauss-Bonnet theorem will give a contribution at the domain boundary. The description of a phase separated (*i.e.*, containing multiple domains) vesicle is therefore more difficult but also more interesting than that of a uniform vesicle. Moreover, as we study in detail in chapter 3, phase separation into domains within the lipid membrane results

in a line tension on the boundaries of those domains. We therefore add an additional energy term which penalizes domain boundaries. The total energy of an axisymmetric vesicle with two domains is then given by

$$\mathcal{E} = \sum_{i=1}^{2} \int_{\mathcal{M}_i} \left(\frac{\kappa_i}{2}(2H)^2 + \bar{\kappa}_i K + \sigma_i \right) \mathrm{d}S + p \int \mathrm{d}V + \tau \oint_{\partial \mathcal{M}} \mathrm{d}l, \tag{2.102}$$

where the line tension τ on the boundary line between $\mathcal{M}_1$ and $\mathcal{M}_2$ plays a role similar to that of the surface tension σ on the membrane area. Together, $\mathcal{M}_1$ and $\mathcal{M}_2$ still form a closed surface. If we locate the boundary at $s = 0$, the energy of each of the bulk parts is given by equation (2.95), but we get additional contributions at the boundary due to the presence of a line tension and a difference in Gaussian modulus. Using the Lagrangian formulation and translating the Gaussian modulus term into a constant contribution (which we ignore) plus a boundary term, we find

$$\mathcal{E} = 2\pi \left[\kappa_1 \int_{-s_b}^{0} \mathcal{L}_1 \, \mathrm{d}s + \kappa_2 \int_{0}^{s_e} \mathcal{L}_2 \, \mathrm{d}s + \tau r_0 + \Delta\bar{\kappa} \cos\psi_0 \right]. \tag{2.103}$$

Here r_0 and ψ_0 are the vesicle radius and tangent angle at the domain boundary ($s = 0$) respectively, $\Delta\bar{\kappa} = \bar{\kappa}_2 - \bar{\kappa}_1$ and the two domains run over $(-s_b, 0)$ and $(0, s_e)$. Colloquially we can refer to the domain boundary as the vesicle's equator and the extrema (at $s = -s_b$ and $s = s_e$) as its poles. The differential equation describing the vesicle shape in each of the bulk domains is still given by (2.94), as follows again readily from the Euler-Lagrange equations. Variation of the free energy (2.103) also gives us the boundary conditions at the domain boundary. By stationarity of $\mathcal{E}$ with respect to variations in r_0 and ψ_0 we obtain the conditions [52]:

$$\lim_{\varepsilon \downarrow 0} \frac{\partial \mathcal{L}_2}{\partial r}(\varepsilon) - \lim_{\varepsilon \uparrow 0} \frac{\partial \mathcal{L}_1}{\partial r}(\varepsilon) = \tau, \tag{2.104}$$

$$\lim_{\varepsilon \downarrow 0} \frac{\partial \mathcal{L}_2}{\partial \psi}(\varepsilon) - \lim_{\varepsilon \uparrow 0} \frac{\partial \mathcal{L}_1}{\partial \psi}(\varepsilon) = -\Delta\bar{\kappa}_1 \sin\psi_0, \tag{2.105}$$

which translate into

$$\lim_{\varepsilon \downarrow 0} (\gamma(\varepsilon) - \gamma(-\varepsilon)) = \tau, \tag{2.106}$$

$$\lim_{\varepsilon \downarrow 0} (\kappa_2 \dot{\psi}(\varepsilon) - \kappa_1 \dot{\psi}(-\varepsilon)) = -(\Delta\kappa + \Delta\bar{\kappa}) \frac{\sin\psi_0}{r_0}, \tag{2.107}$$

where $\Delta\kappa = \kappa_2 - \kappa_1$. The boundary condition (2.106) combines with equation (2.98) to give a condition on the second derivative of ψ:

$$\lim_{\varepsilon \downarrow 0} \left(\kappa_2 \ddot{\psi}(\varepsilon) - \kappa_1 \ddot{\psi}(-\varepsilon) \right) = \left(2\Delta\kappa + \Delta\bar{\kappa} \right) \frac{\cos\psi_0 \sin\psi_0}{r_0^2} + \frac{\sin\psi_0}{r_0} \tau. \tag{2.108}$$

Alternatively, these boundary conditions can be derived by considering force and torque balance [53]. Equations (2.107) and (2.108) tell us that there can be discontinuities in $\ddot{\psi}$ and even $\dot{\psi}$ at a membrane domain boundary if there is a line tension τ between the domains or the bending or Gaussian moduli are not equal in the different domains. These boundary conditions will play a vital role in determining the shape of completely phase-separated membrane vesicles in chapter 4. Their influence on the vesicle shape will provide us with a tool with which we can measure the physical parameters τ and $\Delta\bar{\kappa}$.

Apart from the possibly discontinuous boundary conditions on $\dot{\psi}$ and $\ddot{\psi}$, there are also conditions on $r(s^*)$ and $\psi(s^*)$. Both should be continuous. If $r(s)$ is not continuous at s^* there is a hole in the membrane; if $\psi(s)$ is not continuous there is a sharp kink which carries infinite curvature and therefore infinite energy.